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Insects to Feed the World

Torino

9-12 June 2026

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Foreword

The *Insects to Feed the World* (IFW) conference series has established itself as the leading international forum dedicated to advancing insects as sustainable resources for food, feed, and circular bioeconomy applications. Since the inaugural conference held in Wageningen, The Netherlands, in 2014, the IFW community has steadily expanded, bringing together researchers, industry leaders, policymakers, entrepreneurs and innovators from across the world to address shared global challenges through science, technology and collaboration.

It is therefore a great pleasure to welcome you to the 6th edition of *Insects to Feed the World*, taking place in Torino, Italy, from 9 to 12 June 2026. With its strong scientific tradition, industrial heritage, and internationally recognized food culture, Turin offers an ideal setting for discussing the future of insect production and its role in sustainable food systems and the circular economy.

The scientific contributions collected in this volume reflect the extraordinary evolution and growing maturity of the insect sector. What was once considered an emerging field has become a multidisciplinary scientific and industrial ecosystem addressing critical issues such as food security, sustainable agriculture, resource efficiency, climate change mitigation, waste valorization, and alternative protein production.

A defining feature of IFW 2026 is the strong focus on insect biology as the foundation for scalable and resilient production systems. Numerous contributions explore insect genetics, physiology, nutrition, microbiology, immunity, and reproductive biology, highlighting how fundamental biological knowledge is increasingly essential for optimizing industrial applications. The growing attention dedicated to microbiomes, host–microbe interactions, disease dynamics, and pathogen surveillance demonstrates the sector's awareness that long-term sustainability depends on maintaining healthy and robust insect colonies.

Another major theme emerging throughout the conference is the optimization of insect nutrition and bioconversion efficiency. Research presented in this volume investigates nutrient requirements, dietary modulation, metabolic regulation, substrate valorisation and feed conversion strategies across multiple insect species. These studies reinforce the central role of insects in transforming low-value organic side streams into high-value biomass while contributing to more circular and resource-efficient food systems.

The conference also highlights the increasingly global dimension of insect farming. Contributions from diverse geographical regions illustrate how insect production systems are being adapted to different environmental, economic and social contexts. In particular, the growing interest in decentralized and locally adapted production models reflects the expanding relevance of insects for supporting waste management, feed production, soil health, and sustainable agriculture in both industrialized and developing regions.

At the same time, IFW 2026 demonstrates how rapidly the field is integrating advanced technologies and innovative methodologies. The program includes research on genomics, selective breeding, molecular tools, precision phenotyping, artificial intelligence, computer vision, metabolic modelling and microbiome engineering. Together, these approaches are opening new opportunities for improving productivity, resilience, traceability and sustainability in industrial insect systems.

The conference also reflects the increasing attention being paid to insect welfare, ethical considerations, biosecurity, regulatory frameworks, and societal acceptance. As insect farming scales globally, these dimensions will play a central role in shaping responsible and sustainable development of the sector.

Most importantly, IFW 2026 continues the tradition of fostering dialogue and collaboration across disciplines and sectors. Progress in insects as food and feed depends on the combined efforts of academia, industry, public institutions, investors and society. The diversity of perspectives represented in this conference is a testament to the vitality, creativity, and international character of the IFW community.

We sincerely thank all authors, speakers, reviewers, sponsors, exhibitors and participants for their valuable contributions to IFW 2026 and to this volume of abstracts. Special thanks are due to the organizing and scientific committees for their dedication and commitment in making this conference possible.

We hope that IFW 2026 in Torino will inspire new ideas, strengthen collaborations, and contribute to shaping a more sustainable and resilient future through insect science and innovation.

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Welcome to Torino, and welcome to IFW 2026.

Laura Gasco, Manuela Renna, Ilaria Biasato and Sara Bellezza Oddon
on behalf of the Scientific and Local Organizing Committees

KEYNOTES

Insect health and disease: focusing on the microbiome

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Insects have emerged as a promising new commodity in agricultural production. Certain insects are particularly efficient at bioconverting a variety of organic waste streams into high-quality protein, lipids and other bioproducts such as chitin. Leveraging these insects could help address critical global issues like food insecurity and environmental sustainability. However, intensive animal farming also presents significant challenges. Housing animals in small spaces, at high densities, and in stressful environments, generates ideal conditions for the development and transmission of infectious diseases. Viral, fungal or bacterial infections can affect a vast fraction of the population in a sometimes very short time frame, which can be devastating to the industry. Studying the collection of microbes (or microbiome) harboured by currently farmed insects is therefore critical to understand what a healthy balance of microbes is and how it differs from the diseased state. Characterizing healthy insect microbiomes could aid producers in monitoring colony fitness and minimise environmental stress in rearing facilities, to ultimately accomplish production objectives. Harnessing the knowledge about a specific harmful virus or bacteria, as well as whole microbial signatures associated with health and positive production traits will better prepare us to minimise stress and disease and to promote more resilient insect production systems.

KEYNOTES

Insects aren't failing — they're just succeeding elsewhere

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The narrative that the insect industry is failing is increasingly shaped by the recent setback of ventures in Europe and North America. Yet this perspective obscures a more important reality: insects, particularly Black Soldier Fly (BSF), are not failing, they are shifting toward contexts where their value is strongest. This keynote reframes the discussion by centering low- and middle-income countries (LMICs) as the emerging frontier of BSF innovation. Across much of Africa and Asia, the pressures are immediate and structural. Animal feed accounts for up to 60–70% of livestock production costs, driven by reliance on soybean meal and maize. At the same time, waste management systems are under severe strain, with a large portion of waste left uncollected, and organic materials making up 60–70% of municipal solid waste. These challenges are not isolated, they are deeply interconnected. BSF sits precisely at this intersection. Its ability to convert organic waste into high-quality protein for feed and organic fertilizer, positions BSF not only as a feed solution, but as a climate mitigation strategy, reducing methane emissions from decomposing waste while closing nutrient loops. The resulting frass offers a pathway to restore degraded soils, a critical need in Sub-Saharan Africa where soil fertility is declining, and agricultural productivity is under pressure. Year-round tropical climate conditions reduce the need for energy-intensive infrastructure, while urbanisation and agro-industrial growth concentrate reliable waste streams. These conditions fundamentally shift the economics of BSF, enabling lower-cost and more distributed production models. However, realising this potential requires moving beyond inherited assumptions of what scale means. In LMIC contexts, the priority is not maximum technological control, but systems that are cost-efficient, modular, and embedded within local value chains. This places new emphasis on appropriate technology, decentralized supply chains, and enabling policy environments.

Unlike in Europe, where regulatory constraints, high capital intensity, and centralized scaling models have limited viability, LMICs present an opportunity to rethink the sector. Lower legislative barriers and decentralized systems allow BSF to operate closer to both waste streams and feed demand.

This keynote opens a two-part session combining presentations and panel discussions, exploring both opportunity in LMIC and implementation. Rather than asking why insects are failing in Europe and North America, we should ask where they are working, and why.

KEYNOTES

Insect nutrition, of course

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This talk on insect nutrition starts with an explanation of why this field is relevant. Then, it deals with the concept of feed suitability, and its underlying factors such as palatability, composition, digestibility and nutritional requirements. Firstly, the classical foundation of these aspects is covered as it was explored in the previous century. Then, it deals with the slowly pivoting approach of experimental studies conducted in the last two decades, which has led up to the GIN-TONIC COST Action. Within this Action questions such as what determines feeding behaviour, how do insect digestive systems function and which nutrients are required in which ratios and forms are addressed. Moreover, it calls for the development of standardized methodologies, as currently knowledge is fragmented, sometimes inconsistent, and has varying levels of detail among the different insect species. The field of insect nutrition should combine knowledge on well-developed animal production systems, with an eye for detail to break through discipline and species boundaries. Understanding the nutritional needs of edible insects will improve the valorisation of underutilized by-products and waste and increase the circularity of our food system. Lastly, we set a point on the horizon: what are the long-term goals to develop his field further? What is the low-hanging fruit, and where do we need to focus our efforts to achieve these goals?

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Scientific declaration on insect sentience and welfare

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In a Scientific Declaration on Insect Sentience and Welfare, coordinated by Eurogroup for Animals, scientists and philosophers express support for increasing research in insect sentience, and for the development and implementation of evidence-based welfare measures in insect farming. This Scientific Declaration aims at synthesising the most recent neurobiological and behavioural evidence on insect sentience and welfare. While scientific consensus on insect sentience remains uncertain, a growing body of evidence suggests that certain insect orders, including those of some farmed species, may experience pain. Moreover, various insect species present behaviours indicative of cognitive complexity, including avoidance learning, risk aversion, site-specific grooming of injuries, and protection from further harm. These behaviours, coupled with the presence of homologous nociceptors akin to those in mammals, suggest pain responses in insects. The prevailing focus of animal sentience and welfare research on vertebrates has overlooked the vast diversity of invertebrates, and notably of insects. There is a gap in understanding the cognitive, neurobiological, and behavioural aspects of insect welfare. As the evidence accumulates, there is a need to extend research efforts into insect welfare to ensure their well-being is considered in the developing insect farming sector. Prioritising the welfare of insects necessitates the development of tailored assessment tools. While some best practices, gathered from evidence on black soldier fly, yellow mealworm, and cricket welfare, can be implemented, there is a pressing need for defining precise, species-specific welfare measures. These include considerations such as humane slaughter methods, appropriate anaesthetics, avoidance of pre-slaughter starvation, safe feeding substrates, nutritional support, genetic manipulation prohibition, optimal densities, light conditions for photophobic species, and disease management protocols. Research is lacking on insect welfare, and thus constitutes one of the limitations of this Declaration. Prioritising this research is crucial in developing updated measures based on the Five Domains of Animal Welfare Model (Nutrition, Environment, Health, Behaviour, and Mental Domains), that are comprehensive and species-tailored. The insect farming sector has an opportunity to heed the latest results in insect welfare research and collaborate in developing efficient and implementable welfare best practices.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Operational drivers and management of a disease event in industrial *Hermetia illucens* rearing systems

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Entosystem has operated industrial black soldier fly (*Hermetia illucens*) production systems since 2016 and has valorised recovered retail food waste as larval feedstock since 2019. While biological variability is inherent to large-scale insect production, 2025 marked Entosystem's first sustained disease event impacting operational performance. This contribution presents an industrial case study focused on process-level drivers, mitigation strategies, and remaining uncertainties. The disease was first observed in autumn 2024 and rapidly contained, but re-emerged in spring 2025 and persisted for approximately 10 weeks. A structured process-mapping approach was used to analyse the full rearing cycle, including larval density, feeding steps, and microclimatic conditions. Environmental parameters (temperature, relative humidity) were monitored alongside operational performance metrics. Preliminary microbiological analyses were conducted in parallel, and the investigation is ongoing in collaboration with veterinary specialists. Postulates of Koch have not yet been fulfilled. Disease expression in our system occurred predominantly during late-stage rearing and was consistently associated with an intermediate feeding step involving high larval densities. This step was characterised by elevated temperatures (approx. 46 °C) and low relative humidity. Affected bins showed reduced harvest performance but did not experience mass mortality, typically retaining approximately 30–80% live larvae. Preliminary analyses identified *Paenibacillus thiaminolyticus* as a likely causative agent. This bacterium is ubiquitous and has previously been associated with "soft-rot" disease in mass-rearing facilities in China (She *et al.*, 2023). In our system, removal of the intermediate feeding step resulted in operational recovery and continued production. Despite successful mitigation, observations suggest that the pathogen may persist at low levels and re-emerge under stressful conditions, highlighting the difference between disease suppression and eradication in industrial insect systems. Our findings align with previously reported environmental risk factors, while emphasizing the role of process architecture and density management. This case study underscores the need for the insect industry to collectively address pathogenic threats through improved biosafety frameworks, systematic disease reporting, and closer collaboration with veterinary expertise. Advancing colony health will be essential to ensuring the long-term resilience of *H. illucens* production systems and their contribution to organic waste management and the circular economy.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Feeding adult black soldier flies their preferred foods increases mating activity, egg output, and longevity

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The black soldier fly (BSF; *Hermetia illucens*; Diptera: Stratiomyidae) is reared in the trillions annually. Optimising adult breeding systems is an essential part of productivity and welfare. Although much research has focused on optimising larval diet, current industry standard practice generally involves not feeding BSF adults and providing only water, often due to a misconception that BSF adults cannot eat. However, BSF adults have functional mouthparts and digestive systems, gravitate towards and consume food when it is provided, have been found with digestible materials in their guts in the wild, have sugar-specific gustatory receptors, and live longer when provided with food. This suggests there may be untapped potential in optimising adult breeding systems, for both productivity and welfare, in improving adult feed availability and quality. In this study, we determined what carbohydrates and proteins adult BSF preferred, their preferred ratio of these ingredients, and their preferred concentration of these ingredients in water. Then, we assessed mating behaviour, sex-specific longevity, and egg output when flies were fed their preferred diet v. provided only with water. Flies that were provided with the diet were observed to mate more frequently, laid more eggs on days 2–5 post-eclosion, and lived 4–6 days longer compared to flies only provided with water. Gel-based formulations of the preferred diet may be even more practical to use in industry settings than liquid baits. Altogether, these data add to the growing body of literature demonstrating adult BSF can eat both carbohydrates and proteins and that feeding may benefit their reproductive output and welfare.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Breaking the mortality taboo: case report of a pathogen outbreak from an industrial black soldier fly larvae producer

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Since the early 2010s, the black soldier fly larva (BSFL; *Hermetia illucens*) has been increasingly reared as an alternative feed. Numerous studies praised its ability to feed on a wide range of microbe-rich organic substrates and emphasized that no pathogen of BSFL had been identified. However, BSFL mortality outbreaks were reported over the last few years, often with unclear causes. In late 2024, a similar larval mortality event occurred at the industrial BSFL rearing facility of Agronutris in Rethel, France. Symptomatic larvae were 14-day-old or older and showed brown, flaccid, liquified and sometimes swollen bodies. Affected crates were associated with lower activity, moister frass, strong putrid smell and white exuviae. Transmissibility of the symptoms was investigated by inoculating varying amounts of ground symptomatic dead BSFL to the initial substrate and feeding it to healthy starter larvae. Results showed a positive dose response between inoculation rate and both onset of mortality (5 and 10 days with 0.1% and 0.0001% inoculation rates respectively) and mortality rate after 15 days of rearing (100% and 1% with 0.1% and 0.0001% inoculation rates, respectively). Similar mortality patterns were observed when frass from symptomatic crates was used as the inoculum, whereas no mortality was observed when inoculating healthy ground larvae, rotten cold- or heat-killed BSFL, or frass from healthy crates, suggesting that an infectious agent was present in affected crates. Symptoms were also transmitted when reusing incompletely washed affected crates. Moreover, a heat-treated (80 °C, 15 min) inoculum of symptomatic dead BSFL remained infectious, hinting at the involvement of heat-resistant spore-forming bacteria. Isolation of sporulating bacteria, genome sequencing analyses and *in vitro* feeding bioassays to test *per os* toxicity of different spore preparations, conducted within the FLYPATH research program, identified the causative agent as *Paenibacillus thiaminolyticus*. Based on Agronutris' experience with a successfully identified and contained pathogen, we recommend that insect producers (1) implement a mortality surveillance plan, (2) set-up an investigation task force in case of alerts, including assessment of disease transmissibility, (3) strengthen hygiene practices, particularly crate washing, (4) engage with specialised laboratories capable of identifying insect pathogens, and (5) open up exchanges among insect producers to build a more robust and resilient industry.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Research on the epidemiology and pathogenic mechanism of black soldier fly 'soft rot' disease

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The black soldier fly (BSF) has emerged as a sustainable agent for converting organic waste into high-value insect protein, lipids, and fertilisers, supporting circular economy. However, industrial-scale BSF production faces significant risks from pathogenic outbreaks, particularly "soft rot" disease, which causes high mortality in BSF larvae (BSFL). This study synthesizes advances of our team in the epidemiology, pathological features, and with the aim of mitigating its impact and ensuring secure BSF production for future food and feed systems. Molecular identification of pathogen bacterium was performed using 16S rRNA gene sequencing and whole-genome analysis. Pathogenic traits were assessed under varying temperatures, feed moisture levels, and larvae ages. RT-qPCR was used to detect the expression levels of immune related genes in BSFL after pathogen infection. Multi-step purification process involving salting out, ultrafiltration, and gel filtration chromatography were conducted to identify virulence proteins. The primary pathogens causing BSF soft rot were identified as *Paenibacillus thiaminolyticus*. Disease severity increased with higher temperatures (34 °C), lower feed moisture (50–60%), and younger larvae (4–6 days old). The expression of *Cecropin-like peptide3* and *Hidiptericin-1* genes reached the highest level at 12 h after injection of pathogen bacterium into the abdominal of BSFL, and gradually decreased to the normal level at 24 h and 48 h later. A key virulence protein of the pathogen, Blt-1, was characterised. This is the first report of *P. thiaminolyticus* as an entomopathogen in BSF. The pathogen virulence depends on environmental conditions and larval developmental stage. The identification of Blt-1 provides insights into the molecular mechanism of pathogenicity. These findings collectively underscore the significant biosecurity challenges posed by pathogenic microorganisms in the scaling up of the BSF industry. The elucidation of their pathogenic mechanisms thus provides a crucial scientific foundation for the strategic development of disease-resistant BSF strains.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Viral and bacterial pathogens associated with black soldier fly larval mortality in a French production facility

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Black soldier fly (BSF) rearing has gained popularity as a sustainable protein source for animal feed production. However, high-density rearing conditions may facilitate pathogen transmission and disease outbreaks, possibly leading to mortality and economic losses. While BSF-associated pathogens have been increasingly documented in recent years, comprehensive pathogen surveys in commercial facilities remain limited. This study investigated a French industrial BSF production site experiencing an episode of larval mortality. We employed two complementary approaches to characterise the pathogen community associated with larval mortality. First, targeted molecular screening was performed on asymptomatic and symptomatic larvae sampled at two developmental stages to detect and quantify six previously described BSF-associated viruses (HiIfV, HiSgV, HiLbV, HiSvV, HiInV and HiCV) and *Paenibacillus* 16S rRNA gene. Detection rates varied considerably among pathogens, with HiInV being the most prevalent and HiCV absent from all samples. Second, metatranscriptomic analysis was conducted on the same samples. RNA-seq reads of asymptomatic and symptomatic larvae were mapped against the genomes (WGS) of two bacterial species isolated and sequenced from symptomatic larvae at the same facility: *Paenibacillus thiaminolyticus* and *Bacillus thuringiensis* subsp. *israelensis*}. Differential expression analysis revealed no upregulation at 6 days post-hatching, but significant transcriptional responses at 14 days, with symptomatic larvae showing upregulation of multiple transcripts when mapped against *P. thiaminolyticus*, including genes potentially associated with virulence, while no differential expression was observed against *B. thuringiensis*. These findings reveal a complex pathogen landscape in commercial BSF production, with *P. thiaminolyticus* showing the strongest transcriptional association with larval symptoms, while tested viral pathogens appear unlikely to be primary mortality drivers. The ubiquitous presence of certain viruses regardless of health status, combined with species-specific bacterial responses, underscores the need for integrated pathogen surveillance strategies in the expanding BSF industry.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Green shields for *Hermetia illucens*: boosting the defense mechanisms of black soldier fly via microalgae supplementation

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Feed-induced immunomodulation is an emerging and promising research area that can address the knowledge gaps regarding the nutritional requirements of insect colonies. The black soldier fly larvae (BSFL, *Hermetia illucens*) can convert low-value organic residues into biomass. Thus, the strengthening of the immunity of the larval stage is currently in the spotlight of research. A promising approach is the supplementation of rearing substrates with natural bioactive ingredients. Thus, in this study we designed diets incorporating the microalga *Chlorella vulgaris* at three inclusion levels (1.5%, 5% and 10%) to evaluated whether this approach can enhance the immune response of larvae without compromising crucial traits. Hemolymph was collected from last instar larvae, and its antimicrobial activity was tested against *Micrococcus luteus* and *Escherichia coli*. The strongest suppression occurred at 1.5% microalgae inclusion against *M. luteus*, while no inhibitory activity was detected against *E. coli*. Digestive enzyme activity analysis revealed that the C. *vulgaris* inclusion did not affect midgut physiology, indicating the feasibility of using microalgae as feed additive without adverse effects on midgut digestion. Finally, larval chemical composition was analysed. Larvae fed diets containing *C. vulgaris* exhibited higher lipid content compared to larvae reared on control. Overall, *C. vulgaris* is a promising BSFL feed additive that boosts antimicrobial activity without trade-offs in growth, survival, or digestion, while improving larval nutritional value. This publication is based upon work from COST Action CA23127 — Group on Insect Nutrition: To Open Nutritional Innovative Challenges (GIN-TONIC), supported by COST (European Cooperation in Science and Technology).

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Solinvivirus infection induces cellular antiviral response and reduces the lifespan of adult *Hermetia illucens*

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Viral pathogens pose an emerging threat to the sustainability of insect mass-rearing systems, yet remain understudied in key species like the black soldier fly (BSF, *Hermetia illucens*). Although multiple viral sequences have been reported in BSF, their role in disease has not been established until now. Here, we provide the first *in vivo* characterisation of *Hermetia illucens* solinvivirus (HiSvV), confirming its role as a viral entomopathogen of BSF. Metatranscriptomic analysis of a diseased colony revealed a high viral load attributable to HiSvV. We successfully isolated the virus and developed injection- and oral-based infection assays to investigate replication, tissue tropism, transmission and risk of mortality. HiSvV replicated in inoculated adults, induced premature mortality in flies, and was transmitted both horizontally and vertically. Infected flies also mounted a broad antiviral response, this supported active pathogenesis, even if the small RNA pathways were not activated. These findings establish HiSvV as the first confirmed viral pathogen of BSF and underscore the urgent need for viral surveillance and experimental tools to safeguard industrial insect rearing.

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Dietary protein–carbohydrate ratio effects on growth, digestion, gut microbiota and immunity in black soldier fly larvae

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Black soldier fly larvae (BSFL) are a sustainable source of protein and lipids for animal feed due to their ability to convert organic waste and byproducts into high-value biomass. However, rearing on microbially rich substrates exposes larvae to potential pathogens, making immune competence a key factor in ensuring product quality and safety. This study evaluated the effects of diets differing in protein to carbohydrate (P:C) ratio on larval growth, immune response, and physiological parameters, including midgut functionality, gut microbiota composition, and metabolic markers. Larvae were reared on selected diets and challenged with an *Escherichia coli* (Gram-negative) and *Micrococcus luteus* (Gram-positive) mix. While larval development was not affected by dietary composition, immune responses—evaluated through cellular (haemocyte count and phagocytic activity) and humoral (lysozyme activity and antimicrobial peptide expression) markers—were strongly modified by diet. Larvae fed a protein-rich diet exhibited enhanced immune competence compared with those reared on carbohydrate-rich substrate. Increased haemocyte number and phagocytic activity, together with higher antimicrobial peptide expression and lysozyme activity, were accompanied by diet-dependent changes in metabolic pathway enzyme expression, lactate production, and ATP levels, indicating coordinated allocation of metabolic resources toward immune functions during bacterial challenge. In contrast, the carbohydrate-rich diet supported adequate larval development but was associated with reduced activation of immune and metabolic markers, suggesting preferential allocation toward growth rather than immune defence. The P:C ratio of the rearing substrate also affected the activity of midgut enzymes involved in nutrient digestion and induced shifts in gut microbiota composition. Overall, these findings highlight a trade-off between growth and immune-related metabolic investment in BSFL and provide insights for optimising dietary formulation to balance growth performance, metabolic efficiency, and immune robustness in BSFL rearing systems. This work was funded by MUR (P2022MZAF8) Finanziamento dell'Unione Europea — NextGenerationEU — PNRR Missione 4, Componente 2, Investimento 1.1

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Short-term pupal heat exposure significantly affects black soldier fly life-history traits

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Mass-rearing subtropical insects in temperate regions requires precise control of abiotic conditions that mimic their natural environment, especially temperature, humidity, and light. In black soldier fly (BSF) larvae, the combination of natural crowding, high densities, and elevated metabolic activity can raise substrate temperatures above 50 °C if not counteracted, inducing irreversible stress responses. Heat stress in BSF larvae has recently gained increased interest, but its impact on the pupal stage remains understudied. Pupae may experience heat stress in dense crates when pupation coincides with peak larval activity. Technical failures of aeration or cooling systems can further intensify fluctuations in temperature. Because pupae remain immobile for approx. ten days during metamorphosis, they cannot actively respond to stress. Consequently, lethal effects of excessive heat may not immediately become apparent, leading to significant delays in compensating for the resulting damages and fluctuations in production. To assess the temperature tolerance of pupae and refine estimates of heat stress risk under production conditions, we conducted controlled heat shock experiments simulating short-term thermal stress as might occur during system failure in industrial facilities. Pupae of two age groups, 2 days (T2) and 5 days (T5) post-pupation, were exposed to temperature levels ranging from 44 to 50 °C for 60 min. Life history traits were subsequently monitored, including eclosion success, male:female ratios, adult survival, egg biomass, egg fertility, and the performance of the next larval generation. Both age groups tolerated 1-h exposure up to 46 °C without major negative effects on eclosion. Higher temperatures significantly decreased eclosion success, with T5 pupae responding more sensitively than T2 pupae. At 48 °C, eclosion dropped to approx. 50% in T2 pupae and below 25% in T5 pupae; temperature stress above this threshold was lethal. Eclosed adults differed in sex ratio, with older pupae (T5) generating proportionally more males. This sex bias, combined with reduced adult fitness caused by heat stress, led to less total egg biomass. Moreover, females from both age groups exposed to 48 °C showed extended oviposition periods and delayed oviposition peaks, however, resulting in lower total egg biomass yields. Our results demonstrate that even short periods of heat stress can have intricate effects on BSF pupal development and subsequent reproductive performance, highlighting the challenges of assessing and mitigating damages in large-scale production systems following incidents with uncontrolled increases in temperatures.

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When and where: data insights for identifying temporal patterns in BSF behaviour and biogeographical hotspots

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This presentation gives data insights for the behavioural ecology of the black soldier fly (BSF), *Hermetia illucens*. *Insights for temporal patterns*: Previously it was thought that oviposition behaviour and egg fertility in BSF could not be easily predicted from mating behaviour, due to a complex and nondeterministic mating sequence. However, a cross-correlation analysis of previously collected data revealed that average mating rates were 97.54% correlated with oviposition in traps, if oviposition was lagged by n = 2 days. Similarly, mating was correlated with total egg weights and hatch rates exhibited 60.29% and 75.16% when these were both lagged by n = 3 days. These patterns align with existing knowledge of BSF reproductive dynamics in both captive and wild environments and could be insightful for increasing the predictability of breeding programs. *Insights for biogeography*: Natural history data on BSF remains scarce, meaning the best source of knowledge about wild distributions currently comes from community science records. An analysis of BSF observation records revealed that 158 out of 254 (62.70%) global regions have more than one BSF sighting. From these records, potential BSF hotspots were identified by correcting for biases such as human population density and community science platform user density. Lastly, the ecological niche hypervolume and the phenology of BSF were both modelled using a dataset comprised of these subsampled observation records paired with weather-climate data gathered via web API. Together, these insights will be important for directing future field-based studies.

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Thriving where others fail: the remarkable tolerance of black soldier fly larvae to polluted environments

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The presence of contaminants in organic waste may compromise the efficiency of black soldier fly-mediated bioconversion, potentially affecting insect physiology, as well as the safety and quality of the resulting insect biomass. In this study, black soldier fly larvae (BSFL, *Hermetia illucens*) were reared on substrates contaminated with lead, polyethylene terephthalate (PET), and *Staphylococcus aureus* to assess the effects of heavy metals (HMs), microplastics (MPs), and pathogens, respectively, on insect life-history traits, larval physiology, bioconversion performance, and contaminant accumulation in insect tissues. Among the tested xenobiotics, only HMs induced slight alterations in some physiological parameters, indicating that BSFL responses are strongly dependent on the chemical nature of the contaminant. Notably, lead exposure did not impair larval growth or survival, but affected immune-related markers. Moreover, the complete removal of contaminants by the larvae was observed only for the microbial pathogen, highlighting the recalcitrant nature of HMs and MPs, and raising concerns regarding the use of insect biomass as animal feed or frass as fertiliser. Overall, these findings reveal complex interactions between BSFL and environmental pollutants, providing new insights into their impact on larval biology and bioconversion efficiency. While the ability of BSFL to reduce foodborne pathogens supports their application in waste sanitization, the accumulation of HMs and MPs in larvae and frass underscores critical safety issues. These results emphasize the need for further research to develop effective entomo-remediation strategies and to assess the risks associated with xenobiotic contamination in large-scale BSF production systems. The study was supported by MUR (2020ENH3NZ), FWO (S018524N), and EMBO Short Term Fellowship (8895).

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Black soldier fly metabolomics: what can NMR tell us about the dietary flexibility of the larvae?

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Black soldier fly (BSF, *Hermetia illucens* L.) is widely recognized as an effective agent for the bioconversion of organic residues into high-value biomass. While the performance of BSF under different diets is well documented, the metabolic plasticity that enables this adaptability remains poorly understood. Nuclear Magnetic Resonance (NMR) enables simultaneous identification and quantification of metabolites in complex samples and is widely used in metabolomics profiling, with extensive use in insect research spanning ecology, development and stress responses. In this work, BSF larvae were reared on experimental diets consisting of partial replacement of control (chick feed) with unconventional ingredients: spent coffee grounds and hamburger meat at defined inclusion levels. After extraction, polar metabolites were profiled by NMR within an untargeted metabolomics approach. Over 40 metabolites were consistently quantified, defining a core BSF larval metabolome that includes most proteinogenic amino acids and key intermediates of central metabolism. In addition, diet-specific compounds were identified (e.g. caffeine, trigonelline). Multivariate statistics (PLS-DA) revealed clear metabolic differentiation between control and experimental diets. The metabolites contributing most strongly to this separation (variable importance in projection >1.5), including carnitine, acetylcarnitine and β-alanine, indicated a shift towards enhanced amino acid and lipid catabolism. Collectively, these results demonstrate how NMR metabolomics provides a robust framework to interpret larval performance under different diets, informing dietary optimisation for efficient and sustainable bioconversion.

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Geography and climate shape genetic diversity in black soldier fly populations across native and introduced ranges

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We investigated the ecological drivers of differentiation in the black soldier fly (BSF; *Hermetia illucens*) using 541 samples from 32 populations across the native range via reanalysis of published microsatellite data. Bayesian clustering identified $K = 3$ as the optimal number of clusters, yet also revealed substructure within the clusters. Kriging maps across sub-continents point to major breaks around the Isthmus of Panama and the Andes Range. North and Central American populations were monophyletic. Ordination approaches confirmed three major groups, with spatial structure in higher-order axes, suggesting potential temperate adaptations further North, as well as bottlenecks, island adaptations, or recolonization events in Puerto Rico. Gradient Forest analysis highlighted key climatic and geographic predictors, with cumulative importance values of 0.54 compared to 0.38 for Moran's Eigenvector Maps. A combination of environmental and spatial variables were strong predictors of native population structure. The Southern/Central-South ancestry interface was driven by precipitation variables, while the Northern/Central-South interface was shaped by temperature and seasonality. Applying the model in Africa, where BSF introduced from multiple sources established wild populations, predicted ecological dominance of one ancestry in more humid regions. This prediction aligns with precipitation variables being significant in the native range and also possible resistance to hybridization in West Africa due to ecological adaptation, suggesting success of historic translocations might be non-random. Our results emphasize the combined influence of geography, climate, and history and provide a basis for predicting responses to environmental change across native and introduced ranges. Further research investigating signatures of ecological adaptation at the genome level might be necessary to shed light on specific morphological, physiologic, or behavioural adaptations and their relevance for breeding. Unlike honey bees or silkworms, BSF offers early-stage breeding potential.

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Assessing the genomic diversity of black soldier flies (*Hermetia illucens*) in ten globally distributed strains

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The black soldier fly (BSF, *Hermetia illucens*) has become a key species for sustainable bioconversion of nutrients and an insect-based feed industry is arising. Although performance and growth differences between different BSF strains are characterised, insight into their domestication history and global genomic diversity is still limited. In this study, we investigated patterns of genome-wide diversity and population structure across ten BSF populations representing European lineages (4 populations), African lineages (3 populations) and Oceanian lineages (3 populations). For each population, we sequenced 20 individuals using Illumina 150 bp paired-end whole-genome sequencing and conducted a comprehensive set of population genomic analyses. After quality filtering and variant calling against the BSF reference genome (GCF_905115235.1_iHerIll2.2), we examined within-population diversity, between-population differentiation (F_{st}), potential selection signatures and population relationships using standard diversity metrics such as PCA, admixture inference, and phylogenomic reconstruction. Overall, this study provides an extensive comparative genomic overview of globally distributed BSF populations and offers a foundational resource for breeding, conservation, and bioconversion optimisation efforts.

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The effect of genetic homogeneity in farmed insects

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Climate change has made sustainable agricultural practices increasingly necessary, and as such, mass rearing insects is being investigated as an alternative protein source. Three main species have emerged as leading candidates: the house cricket (*Acheta domesticus*), the yellow mealworm (*Tenebrio molitor*), and the black soldier fly (*Hermetia illucens*). Colony health and productivity are key considerations for mass production. However, genetic diversity loss can increase disease susceptibility and the potential for population reduction and ultimately extinction. The reason is that farming insects likely leads to relaxed selection pressures for some important fitness traits valued in the wild. For example, the selective pressures to maintain a suite of antimicrobial peptides (AMPs), key effectors of insect innate immunity, are metabolically costly, yet maintained through positive selection. This study evaluated genetic diversity among wild, laboratory, and commercial strains in *T. molitor*, *H. illucens*, and *A. domesticus* to understand the correlation between reduced diversity in populations and the role of relaxed selection on genes important for survival in natural environments but less critical in captivity. Analysis of commercial strains revealed a wide range of inbreeding coefficients (F = 0.0–0.38), suggesting different breeding management strategies may minimise inbreeding accumulation. For example, in *H. illucens*, populations showed elevated and variable inbreeding in farmed strains (F = 0.29–0.53) relative to wild-derived populations (F = 0.23–0.24). In *T. molitor*, inbreeding varied widely, with two strains having lower inbreeding (F = 0.0), while other lines derived via experimental manipulations from single-pair matings showed severe inbreeding (F = 0.28–0.36), as expected. Notably, the strain with the greatest inbreeding was a commercial strain (F = 0.38). To identify gene categories potentially undergoing relaxed selection in highly inbred *T. molitor* populations, we performed genome-wide variant burden analyses to assess whether genes associated with particular biological processes accumulated excess variation. Genes associated with circadian rhythm regulation were enriched among those with higher variant burdens in highly inbred and farmed individuals compared to low-inbreeding and wild-derived individuals, indicating circadian regulation may be less critical under controlled rearing conditions. These results highlight trait-specific responses to relaxed selection in mass-reared insects and demonstrate the utility of this framework for assessing genomic vulnerability. Future studies should apply this pipeline to larger population samples and additional species, including other farming-relevant insects, to improve inference and generalizability.

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High-resolution population genetics of *Hermetia illucens* across Australia and Asia for sustainable breeding

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Commercial production of black soldier fly (BSF), *Hermetia illucens*, has expanded globally since the early 21st century. However, the increase in BSF productivity to industrial-scale production levels remains limited. To increase productivity through improved farming and breeding practices, key knowledge of genetic structure and diversity of wild and captive BSF populations is required. This information enables design of sustainable breeding that maximises productivity while minimising genetic loss and inbreeding. Furthermore, it provides useful information on colonising history and genetic connectivity of the species throughout its range. In this study, we investigate broad-scale population genetic structure and diversity of wild Australian and captive Asian BSF across multiple geographic regions. A total of 559 wild Australian BSF larvae were collected from 12 regions across Australia (QLD: Cairns, Townsville, Brisbane, Zilzie; NSW: Sydney; VIC: Melbourne, Bendigo; NT: Darwin; WA: Perth, Broome, Bunbury; and SA: Adelaide), along with additional captive Asian population representatives (n = 63, South Korea, Vietnam and Indonesia). BSF samples (n = 622) were genotyped using a custom Allegro array containing 6000 targeted genome-wide SNPs. Amongst the wild Australian populations, genetic diversity parameters were higher in the northern Australian populations (e.g., Cairns; He = 0.32, PPL = 95%) and lower in southern Australian populations (e.g., Melbourne He = 0.27, PPL = 92%), reflecting possible colonisation history effects and/or differences in contemporary effective population sizes. Several of captive Asian sample locations displayed elevated inbreeding coefficients (FIS) and decreased genetic diversity (e.g., Vietnam: FIS = 0.16 and He = 0.25). Low to moderate levels of genetic differentiation were observed across Australian sample locations, with FST values ranging from 0.03 to 0.32, consistent with an isolation-by-distance population genetic structure model ($r = 0.44$, $p = 0.003$). Principal component analysis (PCA) revealed 4 key genetic clusters, with clear separation among eastern-, western-, northern-Australia, and Asian populations. These patterns reflect colonisation history and current-day genetic connectivity. Overall, these findings not only provide insights into historical and contemporary micro-evolutionary processes shaping Australian BSF populations, but also provide a population-genetic baseline for Australian BSF breeding to ensure long-term sustainability.

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Early sexing with a molecular marker unlocks sex-specific phenotyping in BSF larvae

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In industrial settings, black soldier fly (*Hermetia illucens*) breeding programs aim to improve production traits such as growth rate, development time, survival, and conversion efficiency. However, selection and phenotyping are typically performed on mixed-sex larval cohorts because larvae are not sexually dimorphic. This obscures sex-specific trait expression and can reduce selection efficiency, particularly when males and females differ in performance or when sex ratios drift across timepoints due to sex-specific developmental timing. Here we present a sexing assay based on a molecular marker that enables reliable sex assignment in BSF larvae and thereby supports sex-stratified phenotyping in breeding contexts. We developed PCR-based assays targeting male-specific DNA sequences and validated them across dozens of individuals spanning different life stages and genetic backgrounds. Molecular sex calls matched reference sex assignments (based on adult morphology) with 100% accuracy, and the assays performed robustly using genomic DNA and haemolymph as input. This early sex identification reveals sex differences that are otherwise masked in pooled analyses. In a breeding context, this enables (i) estimating sex-specific genetic and phenotypic variation, (ii) identifying whether particular traits are predominantly expressed in males or females, and (iii) designing selection schemes that maintain adequate representation of both sexes — important for mass selection where females may be larger and phenotyping could otherwise inadvertently bias the breeding population. Overall, early molecular sexing provides a practical tool to improve the precision, interpretability and efficiency of BSF phenotyping and selection.

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Development and validation of a cost-effective SNP genotyping panel for the black soldier fly

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The black soldier fly (BSF; *Hermetia illucens*) has emerged as a key commercial production species due to its fast growth, environmental tolerance, and high nutrient conversion efficiency. As the industry expands, managing genetic stocks under domestication is important for maintaining population health, optimising performance, and enabling selective breeding. Although genomic resources for BSF exist (i.e., WGS and GBS), their high unit cost, limited global utility, and low scalability hinder their use in commercial settings. To address this, we developed a custom Allegro 6000 SNP targeted genotyping panel for BSF, which was then evaluated across Australian and Asian BSF stocks. Using ddRAD sequencing, 15 million SNPs were identified from 191 BSF originating from eight geographically distinct Australian populations. These SNPs were then filtered to produce a high-quality candidate set for Allegro SNP panel design. The resulting 6000 SNP panel was independently validated using 250 BSF from six Australian, one South Korean, and a domesticated Australian population. The targeted SNP assay successfully generated 5517 high-quality, polymorphic SNPs across all samples. The average SNP read depth was 161 across individuals. Technical duplicates demonstrated 97.3% concordance across samples, confirming high reproducibility of the Allegro SNP assay. Preliminary population genetic analyses revealed clear genetic differences, with the panel accurately resolving genetic patterns consistent with Australian BSF colonisation history, contemporary genetic connectivity, and potential effects of captive breeding. Linkage disequilibrium (LD) analyses indicated sufficient genome-wide LD among SNPs, highlighting the panel's utility for quantitative trait loci investigations. Parentage analysis and genomic relationship matrices successfully resolved maternal lineages and sibship, demonstrating applicability in family-based or genomic selection breeding programs. Overall, the 6000 SNP panel provides a scalable, cost-effective, and reproducible platform for routine genotyping in BSF. It can enable population monitoring, support the management of genetic diversity, and inform selective breeding strategies, thereby supporting the expansion of the BSF industry.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Larval weight-based GWAS reveals a sex-specific locus in black soldier fly

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Industrial production of black soldier fly (BSF) larvae is expanding globally. Larval body weight (LBW) at the 5th instar is a key production trait, as this stage is nutritionally rich and optimal for animal feed. Sexual dimorphism in BSF is known to influence growth and yield; however, the genetic basis of larval growth and sex determination remains poorly understood. A genome-wide association study (GWAS) using a custom 6K Allegro SNP array was conducted for LBW based on genotyping 2092 BSF larvae. This GWAS identified a significant SNP associated with LBW and a putative SNP linked to sex. The GWAS, conducted in GCTA using the MLMA-LOCO framework, identified a significant SNP on chromosome 4 (NC_051852.1_166367432_G_A; effect (β) = 10.34 ± 2.26; $p = 5.07 \times 10^{-6}$) along with four SNPs on chromosome 7. Further investigation of the four SNPs on the X chromosome (Chr 7) revealed that SNP NC_051855.1_6352382_A_T exhibited a strictly non-Mendelian inheritance pattern, consistent with a male-heterogametic system (males hemisygous for X) and female-homogametic system. Among the 2092 individuals, 415 were AA, 1677 were AT, and no TT genotypes were observed. Analysis of allele-specific read depth revealed a dosage effect: the AA genotype (putative females) had a mean depth of 272.41x, whereas the AT genotype (putative males) showed reduced depth for allele A (108.89x) and higher depth for allele T (159.62x). Genetic differentiation at this locus was high ($F_{ST} = 0.38$). This pattern was validated in an independent subset of individuals with known phenotypic sex (42 males, 57 females), showing complete concordance between genotype and sex. When chromosome 7 was excluded, and putative sex information was included as a fixed effect, the GWAS was rerun for LBW, recovering the same top association on chromosome 4 (NC_051852.1_166367432_G_A; β = 10.32 ± 2.24; $p = 2.05 \times 10^{-6}$). In addition, a few other loci (p approx. 10^{-4}) were detected across chromosomes, suggesting a polygenic architecture for LBW. Overall, these results provide evidence for a sex-determining locus on the X chromosome (Chr 7) in BSF and indicate a polygenic genetic architecture underlying LBW. Together, these findings provide a genomic foundation for sex identification and inform breeding strategies aimed at improving LBW in industrial BSF larvae production.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

OSDel-BSF: ovary-targeted delivery enables scalable CRISPR editing and RNAi in the black soldier fly

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The black soldier fly (BSF, *Hermetia illucens*) is a rapidly emerging platform for sustainable protein production, yet functional genetics remains constrained by the low throughput and technical burden of embryo microinjection. We developed OSDel-BSF, an ovary-targeted delivery approach that exploits vitellogenin receptor (VgR) mediated uptake to deliver nucleic-acid cargos into developing oocytes following injection into females. To maximise uptake and reproducibility, we optimised injection timing by mapping VgR expression and introducing a pre-eclosion sex-enrichment protocol (yielding 72% females). We assessed OSDel-BSF for heritable genome editing by delivering CRISPR–Cas9 ribonucleoproteins (RNPs) with multiple sgRNAs and quantifying editing outcomes in egg batches. Across 7 sgRNAs, batch-level editing ranged from 11% to 48%. While per-individual efficiency may be lower than direct embryo injection, OSDel-BSF increased the number of edited eggs per handling effort by approx. 120×, enabling substantially higher-throughput screening. Functional effects were observed for developmental targets, including delayed transition to the prepupal stage following Sema1A targeting, and antennal abnormalities accompanied by reduced eclosion after eyeless targeting. To enable non-transgenic functional testing, we extended OSDel-BSF to dsRNA delivery. dsRNA was detected in ovaries hours after injection, alongside increased expression of canonical RNAi components (Dicer2 and Argonaute2), consistent with activation of RNAi machinery. In a proof-of-concept trehalase assay, OSDel-mediated dsRNA delivery produced measurable knockdown (25% in one experiment and 50% in a second, relative to controls). Ongoing work is focused on calibrating dsRNA dose, timing, and target selection to maximise and standardize knockdown. Overall, OSDel-BSF provides a scalable platform for both heritable CRISPR editing and RNAi-based gene silencing in BSF, supporting higher-throughput target screening and strain-development workflows.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Unveiling host-microbe interactions critical dynamics in black soldier fly: from hatching to bioconversion

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The black soldier fly serves as a cornerstone of the circular bioeconomy, yet its industrial efficacy is fundamentally governed by the insect's "holobiont" status—an obligate reliance on microbial symbionts. While the role of gut microbiota in waste reduction is acknowledged, the ontogenetic continuity of these interactions from embryonic development to larval bioconversion remains underexplored. This study integrates findings from two distinct phases to elucidate how BSF orchestrates microbial communities to overcome developmental barriers (hatching) and optimise metabolic performance (waste valorisation). We analysed embryonic microbial succession (0–72 h) via sterilization and sequencing to identify hatching promoters. Subsequently, a Germ-free (GF) larval model across three wastes was established to test "host-mediated environmental recruitment." A synthetic consortium of functional gut symbionts was then constructed to validate its impact on larval development and bioconversion efficiency. Our results demonstrate that BSF performance depends on continuous microbial symbiosis. Hatching proved to be an obligate, microbe-dependent process; surface sterilization reduced hatching rates from 77.1% to 33.7%, whereas re-inoculation with a synergistic *Bacillus* and *Staphylococcus* co-culture restored rates to 83.4%. These bacteria putatively secrete chitinases and proteases to degrade the chorion, acting as an "extracorporeal digestive organ" facilitating eclosion. Transitioning to the larval stage, BSF actively recruited specific environmental microbes to compensate for sterile conditions. A host-selected synthetic consortium (*Enterococcus*, *Bacillus*, and *Lactobacillus*) significantly rescued GF larval phenotypes, most notably in chicken manure. Compared to controls, this consortium increased survival by 72.1%, biomass by 193.6%, and feed conversion efficiency by 439.6%, while shortening development time by 62.4%, confirming the efficacy of host-mediated recruitment. The BSF lifecycle is inextricably linked to microbial symbiosis. From the enzymatic "softening" of the eggshell during hatching to the selective enrichment of metabolic partners for waste degradation, the host actively shapes its microbiome to adapt to environmental constraints. These findings underscore the potential of developing stage-specific probiotic formulations — targeting both egg hatching synchronization and larval waste conversion efficiency — to standardize and upscale industrial insect farming.

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Phenotypic plasticity and microbial signal: unraveling the *Hermetia illucens* response to long-term dietary shifts

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Black soldier fly larvae (BSFL) are increasingly recognized as a promising and sustainable solution for upcycling low quality side streams into high-quality protein, but the underlying genetic and environmental mechanisms influencing their performance remain poorly understood. This study investigated the effects of cross-generational dietary changes on BSF performance and larval gut microbiome composition. We used BSF lines that had been maintained on either low-quality (wheat bran) or high-quality (chicken feed) diets for 30 generations (parental diets) and subjected them to dietary shifts across three generations (F1-F3). Results revealed improvement of growth and reproductive traits when larvae were raised on a high-quality diet, regardless of the parental diet. We also identified subtle effects of parental diet on offspring growth traits when larvae were fed the same diet. However, in most cases this latter effect was transient and disappeared in the F3 generation, suggesting a strong environmental influence on the performance traits. The gut microbiome of BSFL was significantly shaped by dietary changes, with a marked change in composition and generally higher microbial diversity when larvae were reared on wheat bran diets. In addition, a small but significant trans-generational effect of wheat bran parental diet was detected, revealing a potential signal of host/microbiome adaptive response to this diet. However, this potential link between gut microbiome composition and BSF larvae performance was not evident in the traits measured here. These findings provide valuable insight into the effects of diets on fitness components and the microbiome in BSF and offer potential avenues for optimising farming of this species.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Determining the gut microbiota composition of the yellow mealworm *Tenebrio molitor* in its industrial rearing environment

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Over the past decade, insects have received increasing interest as an innovative and sustainable source of alternative protein for both human food and animal feed. The yellow mealworm (*Tenebrio molitor*) is one of the most promising candidates due to its high protein content and ease of breeding and feeding. As in all livestock sectors, understanding and managing the microbiological aspects of mealworm production is crucial to ensuring quality and profitability. To date, the scientific literature is limited regarding mealworm gut microbiota under large-scale rearing conditions, as most studies have been conducted under controlled laboratory conditions. Thus, this study aimed to characterise the microbial communities present in the gut microbiota of *T. molitor* under industrial rearing conditions. Regular sampling of insects and substrates was performed throughout the life cycle of the insect under large-scale production conditions, including young larvae, fattened larvae, pupae, and beetles. In parallel, welfare indicators and zootechnical performance were rigorously monitored over the insect full cycle. To analyse the gut microbiota of the collected samples, DNA extractions followed by high-throughput sequencing were performed. Two complementary strategies were used: an exploratory shotgun metagenomic approach and a targeted 16S rRNA metabarcoding. The sequencing data are currently under analysis, but the preliminary results provide initial insights into the microbial composition of *T. molitor*. Among all microorganisms detected in *T. molitor* larvae, bacteria represent 94%, while eukaryotes, viruses, and archaea account for 3%, 2% and 1%, respectively. Focusing on the bacterial population, the predominant phyla are Bacillota, Pseudomonadota and Bacteroidota. The data generated in this study may offer new perspectives on the interactions between the insect's gut microbiota, its zootechnical performance and the environmental conditions of industrial rearing.

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Beyond linear nutrition: evidence for circular, microbially driven nutrient dynamics in black soldier fly larvae

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There is significant interest in defining the nutritional requirements of black soldier fly larvae (*Hermetia illucens*), yet many foundational principles of classical animal nutrition do not fully translate to this system. Traditional nutrition models assume a linear flow of nutrients from feed to animal biomass, with excreted wastes representing irreversible losses. In contrast, BSF larvae develop within microbially dynamic substrates in which nutrient pools are continuously transformed by resident microbiota. Larval feeding rapidly depletes specific limiting nutrients, imposing selective pressure on the substrate microbiota and favouring taxa capable of synthesizing, mobilizing, or recycling those nutrients. Larval waste products — including exuviae, uric acid, and partially digested feed — can be converted in situ into microbial biomass and subsequently re-enter the larval food supply. Microbial communities thus actively modulate nutrient availability and can partially compensate for nutritionally deficient substrates. Such feedback loops can confound controlled nutritional experiments, as larvae sometimes exhibit robust growth on experimental substrates intentionally formulated to be inadequate. This presentation examines how such microbial dynamics challenge conventional nutritional models and highlights the need for nutritional methods that explicitly account for larva–microbe–substrate interactions. Using an axenic (germ-free) rearing model that does conform to classical nutritional assumptions, we determined the "true" lysine requirement of BSF larvae using substrates with graded supplementation levels (0, 0.05, 0.1, 0.2, 0.5, 1, 2, or 3% lysine added). Isonitrogenous controls were prepared using mixed amino acids from hydrolysed casein at the same levels, and larvae were sampled 20 days after egg collection. Significant dose-dependent differences in growth were observed in axenic larvae ($p = 0.007$), whereas lysine depletion/supplementation did not significantly affect larval growth in conventional (i.e. non-axenic) larvae under the same conditions. Together, these results demonstrate that while axenic systems can reveal the absolute biological nutrient requirements of BSF larvae, the relevance of these requirements for practical substrate formulation may be limited in microbially active environments. Accounting for circular, microbially mediated nutrient flows represents the next frontier in developing accurate and useful nutritional models for *H. illucens*.

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Incidence and detection of spore forming bacteria in mass-reared black soldier fly larvae

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The larvae of the black soldier fly (*Hermetia illucens*) (BSFL) can grow on a wide range of wastes and byproducts, differing in nutritional composition and pH and representing the optimal growing environment for many microorganisms that might represent a risk for human or animal health. BSFL are known for their capacity to reduce some pathogens. However, their efficacy in eliminating spore-forming bacteria is less well documented. In this study we describe *Paenibacillus thiaminolyticus*, a Gram+ spore-forming bacteria, isolated from symptomatic BSFL in France, and discuss its interactions with the growing media and the host. First, the evaluation of *P. thiaminolyticus* ecology in diet and its persistence in BSFL was evaluated in order to estimate the risk of contamination and pathogens persistence during insect rearing. Then, challenge tests using pathogen inoculation into the diet were performed to assess the virulence of *P. thiamynolitycus* on BSFL growth, survival and eventual capacity to reduce *P. thiaminolyticus*, depending on spores or vegetative form. In laboratory conditions, the infected insects exhibited symptoms such as brownish colour, softening, liquefaction, off flavour, death, decay of the larvae, cannibalistic behaviour, and arrested or delayed development, resulting in reduced larval weight, pupation and adult emergence. However, on the industrial site, symptoms were sometimes more severe, with growth arrest, softening, death, and decay of larvae. During the challenge tests, the presence of the *P. thiaminolyticus* strain in both BSFL and diet was verified with specific molecular detection probes as well as by using fluorescence and antibiotic labelling genetic techniques. This study describes a new spore-forming BSFL pathogen, a *P. thiaminolyticus* strain, and underscores the importance of developing good manufacturing practices (GMP) guidelines and detection methods for spore-forming pathogens in rearing equipment, feeds and larvae to preserve insect health in mass rearing environment. Key words: food and feed safety, insect health, *Paenibacillus* spp., pathogen detection, *Hermetia illucens*, insect rearing, microbial risk

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Histopathology studies in black soldier fly larvae (BSFL) morbidity and mortality cultured in a laboratory sized colony

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Black soldier fly larvae (BSFL, *Hermetia illucens*) are widely regarded as resilient insects suited to large-scale bioconversion systems; however, their health is influenced by a range of potential pathogens and substrate-dependent risks. Early assessments reported no major natural outbreaks in commercial farms and noted that BSFL show relative resistance to many entomopathogens, despite experimental infections with bacteria, fungi, nematodes, protozoa, and viruses. As global BSFL production expands, the likelihood of pathogen emergence increases, highlighting the need for proactive monitoring and biosecurity. This study assessed histopathology as a diagnostic tool for identifying BSFL disease processes. Histopathology enables detection of structural tissue changes caused by pathogens, toxins, environmental stress, or physiological disruption. Because insects rely primarily on innate immunity, tissue-level alterations often provide early and reliable indicators of disease. During BSFL culture for full-fat insect meal used in fish trials, episodes of tray morbidity and complete mortality occurred. Larvae were fixed in 10% neutral buffered formalin, Davidsons fixative, or Bouin's variants, processed for wax embedding, sectioned, stained with H&E, and examined under light microscopy. The goal was to document insect-specific causes of morbidity and mortality, including organ pathology and in situ pathogen presence. This approach parallels diagnostic methods used in intensively farmed arthropods such as molluscs and crustaceans. Granulomatous inflammation and coagulative necrosis of tissues such as the gastrointestinal tract and coelomic lining were observed, with bacterial agents commonly identified. BSFL are robust but not immune to disease, capable of experimental infection and susceptible to metabolic disruption under stress. Histopathology provides essential insight into the structural impacts of infection and environmental challenge. As industrial BSFL production accelerates, maintaining colony health will rely on accurate diagnostics, pathogen surveillance, and improved understanding of BSFL responses to infectious and non-infectious stressors.

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Profiling the resistome and virulome of the *Hermetia illucens* midgut microbiota

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The spread of antimicrobial resistance has become a crucial global health threat with agricultural practices and livestock production contributing greatly to the spread of antibiotic resistance genes (ARGs) in the environment. However, the spread of ARGs is not the only challenge facing the agri-food sector which requires urgent innovation for the adoption and development of more sustainable processes. The larvae of the black soldier fly (BSFL) *Hermetia illucens* are mass-reared to produce animal feed and fertilisers, yet the resistance and virulence encoded within their midgut microbial communities remain underexplored. To elucidate the risk associated with their use, the profiles of antibiotic resistance genes (ARGs), virulence factors (VFs), and plasmids were predicted from the midgut bacterial community of BSFL. Shotgun metagenomic sequencing revealed the presence of genes encoding resistance against 26 classes of antibiotics, and genes encoding virulence via nine mechanisms of which *Pseudomonadota phylum* taxa were the dominant contributors. Three horizontally-mobile ARG-encoding plasmids were additionally identified. Reconstruction of metagenomes allowed high-resolution taxonomic classification of taxa harbouring multiple genes for resistance and virulence, including a Pseudomonas species with 42 VFs and seven ARGs. Eight unique species of bacteria demonstrated growth on antibiotic containing agar plates inoculated with the BSFL midgut contents. Altogether, this analysis represents the most comprehensive profile to date of the midgut microbiota resistome of BSFL. It will provide both a valuable tool to the research community working on BSF larvae, and for the assessment of the antibiotic resistance risk posed to the environment and to public health through their use.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Black soldier fly immunity shapes the surrounding microbial community via antimicrobial peptides

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Insects rely on antimicrobial peptides (AMPs) as a central component of their innate immune system. The black soldier fly (BSF), a detritivorous insect that develops in microbe-rich substrates, is known to produce a diverse repertoire of AMPs. Here, we investigated whether AMP production by BSF larvae extends beyond host defence to influence the microbial composition of the surrounding environment. We show that BSF larvae produce AMPs in the gut and release them into the rearing substrate. These secreted peptides selectively modulate the microbial community, promoting the growth of certain fungi such as *Candida tropicalis* while suppressing others, including *Aspergillus niger*. This AMP-driven shift in microbial composition is associated with changes in larval performance and may contribute to the exclusion of potentially pathogenic microorganisms from the rearing environment. Our findings suggest that insect immunity can function as an ecological driver shaping microbial communities in insect-rearing systems, with implications for understanding insect–microbe interactions and for improving the stability and safety of industrial BSF production.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Common garden study: stage- and sex-specific trait divergence in black soldier fly lines with contrasting diet histories

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The black soldier fly (*Hermetia illucens*, BSF) is widely used in organic waste bioconversion, yet performance varies substantially among strains maintained under different long-term rearing regimes. To examine how long-term dietary history shapes BSF performance across life stages, we conducted a common garden experiment using eight genetically distinct BSF lines maintained on contrasting diets over multiple generations. Each line was reared on both its historical diet and a compositionally distinct novel diet, allowing direct comparison of larval, pupal, and adult traits under standardised conditions. Larval growth and survival differed strongly among lines and were generally highest when individuals were reared on their historical diet. However, these patterns were not consistently reflected at later stages. Pupal development time and eclosion success showed weak or variable correspondence with larval performance, and adult body size and lifespan often contrasted with larval outcomes. Trait divergence was further sex-specific. Females showed marked differences in ovarian investment between diets, whereas males exhibited relatively stable investment in testes and accessory glands across rearing conditions. These results indicate that BSF lines with contrasting dietary histories differ across developmental stages and between sexes, and that patterns observed at the larval stage do not consistently extend to adult reproductive traits.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Stage-specific feeding effects on mealworm growth, survival and reproduction: evidence from two case studies in Uganda

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Optimising diets across developmental stages is crucial for improving mealworm farming, particularly in systems relying on agro-byproducts. This study evaluates stage-specific feeding strategies on growth, survival, pupal output, and adult reproduction at two East African farms: Bobo Eco Farm and Kyaka II Refugee Settlement in Uganda. Data from 2024–2025 were analysed, including controlled feeding trials at Bobo Eco Farm and routine KPIs from both sites. Larval diets tested were wheat bran (WB), maize bran (MB), and chicken feed (CF) in different combinations. Growth rate, development time, body weight (ABW), survival, and egg production were assessed. For adults, four diets were tested: MB, WB, 50% MB + 50% WB, and 40% MB + 40% WB + 20% CF. Moisture was applied to some replicates to examine its effect on egg laying. Larval experiments included stocking densities (6000–10 000 M/w), wet vs. dry feeding, and finisher diets (WB + CF). Larval diet had a strong impact on performance. In pre-split larvae, 80% MB + 20% CF reduced development time by approx. 50% and increased daily biomass gain six-fold compared to 100% WB. Finisher diets with 20% CF accelerated pupation and increased pupal size. Adults fed 20% CF laid 4–5 times more eggs and had higher survival than those on other diets. Moisture did not affect egg oviposition. Lower stocking densities (6000 M/w) led to faster development compared to higher densities (10 000 M/w). Routine wet feeding resulted in better survival and faster development than dry feeding or refeeding. At Bobo Eco Farm, pupal ABW stabilized at 125–135 mg, but yields declined (5–12 kg/week), and survival to adulthood was approx. 52%. Egg-to-split larval survival improved from <20% to approx. 53%. At Kyaka II, adults produced 2–5 times more eggs per tray (24 000–35 000/week), with larger pupae (approx. 150 mg ABW), but egg-to-larvae survival was low (2–10%). Stage-specific diets enhance mealworm growth and reproduction. MB and CF diets improve larval growth, while 20% CF in adult diets increases egg production. Lower stocking densities and wet feeding optimise development. Discrepancies between trials and farm outcomes suggest external factors may influence results. Diet optimisation based on developmental stage improves mealworm productivity. These findings have direct relevance to commercial insect farming, showing that targeted feeding strategies enhance performance while reducing feed costs.

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From manual measurements to automated precision: a novel predictive phenotyping method for black soldier fly larvae

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Efficient and accurate phenotyping is essential for optimising black soldier fly (BSF, *Hermetia illucens*) production, yet traditional manual measurement protocols are time-consuming and limit data collection. Here we present the development and implementation of an automated phenotyping pipeline for BSF larvae, resulting in significant improvements in throughput, data quality, and analytical capabilities. Initial manual protocols, involving random sampling and calliper-based measurement of larval length and width, required approximately one hour per group and restricted phenotyping frequency to two or three times per week. To overcome these limitations, a series of automation solutions were evaluated, including infrared-based counting systems and adapted vibration-detection seed counters, which provided incremental time savings but were constrained by larval size variability. A substantial advancement was achieved through the integration of an image analyser, customised for BSF larvae. This system reduced processing time to under 10 minutes per group and enabled daily phenotyping. Consequently, the dataset expanded from several hundred to over one million entries within a year, facilitating comprehensive developmental analyses. Leveraging this dataset, three-dimensional predictive models were constructed to estimate larval volume and weight based on corrected morphometric parameters. These models demonstrated a very high correlation with actual weights, confirming their robustness and reliability. The automated pipeline now enables high throughput monitoring of larval development, early sex differentiation, and rapid identification of rearing issues. Additionally, the methodology supports advanced applications such as macromolecule concentration estimation, yield-related trait differentiation, and enhanced nutrient profiling. Current efforts focus on further refining predictive models and extending automation to egg-laying, hatching, and reproductive monitoring through image-based analyses in controlled environments.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Tracking and classifying black soldier fly larval group movement using computer vision

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Black soldier fly larvae (BSFL) are commonly reared on organic waste streams as a replacement for conventional, less sustainable protein sources in feed for livestock. Organic waste streams can introduce challenging conditions that act as stressors for BSFL, potentially leading to altered movement and group behaviour. Monitoring such behavioural changes may help to identify stressors and address growing concerns regarding insect welfare, while also supporting the optimisation of rearing conditions. However, current BSFL performance metrics are largely limited to end-point measures such as protein yield or feed conversion efficiency and lack the temporal resolution needed to detect behavioural anomalies. This study presents an imaging system, consisting of a camera and a computer vision algorithm, to continuously monitor and classify BSFL movement patterns. The imaging pipeline was applied to ten complete rearing cycles and compared to conventional weight measurements. Subsequently images were analysed using a frame-comparison-based computer vision pipeline to track and classify group-level larval movement. Using this pipeline, we were able to track and compare recurring, development-dependent movement patterns in ten different BSFL life cycles. By comparing high-level movement features with larval growth curves, weight peaks were correlated to activity patterns. This research provides novel insights into movement patterns under standard BSFL rearing conditions and sets a foundation for future work to investigate behavioural changes from suboptimal rearing conditions. Furthermore, it can also give insight into how the growth peaks and weight gain in BSFL rearing can be predicted in a cost-efficient and non-invasive way.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Review of larval performance and composition data of *Tenebrio molitor* for predictive model development

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Tenebrio molitor represents a promising insect species for sustainable protein production to meet increasing protein demand driven by population growth. Its rearing is integrated into circular economy systems that valorise agri-food by-products as feed ingredients. However, feed supply often fluctuates in quantity, composition, and availability over time. This variability creates a need to anticipate the effects of diet composition on larval performance and composition. The objective of this study was to compile and harmonize data from the scientific literature to develop a structured database suitable for meta-analysis and to derive predictive models for key production variables. A data-driven review was conducted to identify studies reporting both dietary composition and relevant response variables. Selected articles included data on larval composition (moisture, protein, fat, carbohydrates, ash) and larval performance metrics (growth, development time, feed conversion efficiency, efficiency of ingested food, nitrogen conversion efficiency). In addition to nutritional variables, non-nutritional factors such as strain, temperature, relative humidity, photoperiod and rearing density were recorded and considered in the analyses. Following screening, a total of 37 articles reporting larval composition and 32 articles reporting larval performance met the inclusion criteria. Articles were primarily excluded because nutritional data of the diet were missing or incomplete, and because data were presented exclusively in graphical form without numerical values provided in the text or supplementary materials. Extracted data were then standardized and transformed to ensure comparability across studies and integrated into a unified database. The resulting dataset enabled the application of meta-analytical approaches and multiple linear regression to quantify relationships between dietary inputs and larval responses. Total nitrogen, ash, fibre and lipids were identified as the main explanatory variables used to model multiple response variables related to larval performance and nutritional composition. This work demonstrates that systematic compilation and harmonization of published data can support the development of predictive models linking diet composition to key production outputs in yellow mealworms. The resulting database provides a foundation for decision-support tools aimed at optimising insect diets.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

The carry-over effect of developmental temperature on black soldier fly adult fitness

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The black soldier fly (BSF, *Hermetia illucens*) is a vital resource insect for organic waste bioconversion and feed protein. Its minimum developmental temperature is approximately 10 °C, with 30 °C being the optimal temperature, while 20 °C as a suboptimal temperature is in a range where BSF can maintain survival and reproduction. The impacts of thermal acclimation on BSF fitness and the underlying molecular mechanisms remain unclear. This study investigated the effects of two acclimation temperatures (e.g., 20 °C and 30 °C) on BSF fitness-related traits (e.g., growth, thermal tolerance, body composition, and oocyte quantity/quality) and explored regulatory mechanisms via multi-omics analysis. Results showed that 30 °C-acclimated individuals had higher weights at all stages, lower prepupal weight loss, and smaller adult sexual dimorphism than 20 °C-acclimated ones. Compared with 30 °C, BSF reared at 20 °C significantly prolonged median developmental duration and increased developmental asynchrony. For thermal tolerance, 20 °C-acclimated adults exhibited significantly enhanced cold tolerance (e.g., shorter chill coma recovery time) but reduced heat tolerance (e.g., lower heat knock-down temperature). Nutritionally, thermal acclimation affected adult lipid content but not protein content. For potential fecundity, robust linear regression and mediation analysis showed female body weight positively affected oocyte number, and the acclimation temperature effect on fecundity was fully mediated by female body weight (e.g., no direct temperature effect after controlling for weight); however, notably, most 20 °C-developed females failed to achieve normal oocyte vitellogenesis. Multi-omics analysis revealed potential molecular pathways underlying these phenotypic changes. In conclusion, thermal acclimation reshapes BSF adult fitness by regulating development rate, body weight, thermal tolerance, lipid metabolism, and reproductive potential. This study provides critical insights into BSF thermal adaptation strategies with trade-off effects and a theoretical basis for optimising mass rearing conditions.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Fungal contributions to black soldier fly performance: metabolic mechanisms and implications for rearing optimisation

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Improving the efficiency and stability of black soldier fly (BSF) production systems requires a better understanding of the biological factors shaping larval performance. While bacterial contributions to insect nutrition have been widely explored, the role of fungi in BSF rearing systems remains largely overlooked. Here, we investigated the nutritional and metabolic interactions between BSF larvae and associated fungi. We identified *Candida tropicalis* as the dominant fungal species in household compost systems colonized by BSF. Supplementation of larval diets with *C. tropicalis* significantly increased larval weight, indicating a positive contribution to performance. To elucidate the mechanisms underlying this effect, we examined whether fungal contributions occur directly through larval consumption or indirectly via fungal modification of the feeding substrate. Larvae exposed to *C. tropicalis* showed increased fatty acid biosynthesis, associated with elevated levels of palmitic and myristic acids that are abundant in fungal biomass. In addition, the presence of *C. tropicalis* in the substrate increased larval levels of essential amino acids (threonine, leucine, and isoleucine), suggesting improved nutrient availability mediated by fungal activity in the substrate. These results support two complementary mechanisms by which fungi enhance BSF performance: direct nutritional contribution and indirect improvement of substrate quality. Our findings highlight fungi as a promising, yet underutilised, lever for optimising BSF rearing systems, with potential applications in improving productivity, robustness, and consistency of insect-based feed production.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Industrial valorisation of dairy, tomato and chili processing by-products using black soldier fly larvae

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Converting organic waste into high-protein biomass via the black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) is a key component of circular bioeconomy strategies. However, maintaining consistent industrial performance requires precise diet formulation to manage waste variability. At nextProtein, more than 100 feed ingredients have been screened to bridge the gap between heterogeneous waste streams and production stability. This study evaluates the integration of dairy (yogurt), tomato (concentrate), and chili (harissa) processing residues into BSF larval diets. A stepwise validation protocol was employed, transitioning from lab-scale to industrial pilot tests. Rearing cycles lasted 7 to 10 days. We tested inclusion rates of 15–30% for dairy, 10–21% for tomato, and 4–20% for chili wastes. Performance was measured via larval survival rate, biomass yield, feed conversion ratio (FCR), and residence time, with 30–50 replicates per condition to ensure statistical robustness. Quantitative analysis revealed that dairy wastes significantly boosted efficiency; inclusion rates of 15–30% (fresh matter) resulted in survival rates >94%, a 15–25% increase in biomass yield, and an FCR improvement of up to 20%. Notably, larval residence time decreased from 9 to 7 days. Tomato residues (10–21% inclusion) maintained high survival rates (93–95%) and delivered a 25% increase in yield with 8 day cycles. Finally, chili wastes (4–20%) proved highly compatible, supporting >95% survival and a 15% biomass increase. The results highlight that these wastes are not merely fillers but functional ingredients. The rapid growth observed with dairy by-products suggests a high bioavailability of nutrients that compensates for other waste streams. Tomato residues appear to contribute to stable growth and biomass accumulation, likely due to their balanced carbohydrate and fibre content supporting both feed structure and larval ingestion. The successful integration of chili residues, which contain capsaicinoids, suggests a high metabolic resilience in BSF larvae. This study demonstrates that dairy, tomato, and chili processing wastes can be effectively upcycled at scale. By optimising formulations, we achieved significant gains in FCR and reduced production cycles. These findings support the viability of BSF-based systems to transform diverse agro-industrial side-streams into reliable protein sources, enhancing the overall efficiency of circular waste management.

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Ultrasonic processing effects on the structural and nutritional characteristics of black soldier fly protein

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Ultrasonication has emerged as a viable method for modifying protein structure and improving functionality in food applications. This study examined ultrasound-assisted alkaline extraction to enhance the structure and nutritional features of black soldier fly (BSF) larvae proteins, as edible insects increasingly emerge as sustainable dietary ingredients. BSF samples were sonicated at 300 W for 0, 10, 20, 30 and 40 min. Sonication significantly increased protein recovery (56.14% to 77.07%, $p < 0.05$) and lightness (L^*) (27.3 to 35.8, $p < 0.05$). Free sulfhydryl content rose initially but declined with prolonged exposure. Secondary structures shifted, with α-helix decreasing (36.2% to 27.5%) and β-sheet increasing (23.4% to 32.1%, $p < 0.05$). Functional properties, including water and oil binding capacities and solubility, improved significantly ($p < 0.05$). However, excessive sonication reduced these enhancements. These results demonstrate that controlled ultrasonication can effectively tailor BSF protein functionality, depending on protein type, solution, and treatment parameters.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Broodstock: size is fine, diet is better — a production-scale reality

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Black soldier flies (BSF) are widely recognized as effective agents for organic waste bioconversion. At Entosystem, BSF are reared on grocery store and industrial food-waste streams, including broodstock. While breeder size is known to correlate with reproductive output, the diet used to achieve this size also influences fecundity. During 2025, two broodstock diets were applied sequentially at production scale, supporting the daily production of 1–3 kg of neonates. One diet consisted of a food-waste-derived slurry, while the other was a grain-based formulation excluding food waste. Pupal mass and egg production were monitored continuously under commercial operating conditions. Overall, pupae reared on the grain-based diet were also smaller than those reared on the slurry-based diet (128 mg vs. 140 mg, respectively). However, when comparing multiple periods in which mean pupal mass was equivalent (138 mg), females originating from the slurry-based diet produced approximately 25.3% (about 4 mg) more eggs per female. At production scale, this difference corresponded to an increase of 28%, more than 1 kg of eggs per day. The two diets differed, notably in lipid content, which was higher in the food-waste-derived slurry and may explain the difference in fecundity. These production-scale results indicate that broodstock fecundity in BSF is influenced not only by body size but also by diet composition and this effect can be substantial. We hypothesize that increased dietary lipid availability contributed to enhanced reproductive output. In conclusion enables efficient bioconversion, food waste-based substrates appear to meet the biological needs of BSF breeders and constitute a nutritionally suitable food source for supporting reproductive performance.

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Dietary protein level regulates nutrient composition dynamics in black soldier fly larvae across the life cycle

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Black soldier fly larvae (BSFL) are an alternative protein source; this study tested whether dietary protein alters nutrient accumulation magnitude without affecting its developmental timing. Larvae were reared under controlled conditions on three isoenergetic diets differing in crude protein content: CPlow (10%), CPmed (15%) and CPhigh (20%), with a standard Gainesville diet as control (n = 6 per diet). From six days post-hatch (days of life, DOL), larvae were maintained at 27 °C and 55% relative humidity. Performance parameters were recorded, and individuals were sampled across developmental stages (6–16 DOL, prepupa, pupa and adult) to quantify protein, lipid, chitin and ash masses. Survival to the prepupal stage was high across all treatments (91.1–94.6%). Total biomass gain in fresh matter was maximised in the CPmed group (2.31 ± 0.2 kg), exceeding the lowest value observed in CPlow larvae (1.66 ± 0.2 kg) by 39% ($P < 0.05$). Total development time from 6 DOL to adulthood was longest under protein-limited conditions (23.3 ± 0.3 days) and shortest in the high-protein group (20.2 ± 0.3 days), representing a reduction of 3.1 days ($P < 0.05$). Prepupal body length was greatest in CPmed larvae (13.3 ± 1.2 mm), exceeding CPlow larvae by 24%, with similar patterns observed in adult females and males. Protein mass increased rapidly during larval growth and peaked at the prepupal stage, reaching a maximum of 26.3 ± 0.9 g dry matter (DM) in CPhigh prepupae compared with 19.8 ± 0.8 g DM in CPlow prepupae (+33%; $P < 0.05$). In contrast, lipid deposition was enhanced under protein limitation, with prepupal fat mass highest in CPlow 19.1 ± 0.9 g DM) relative to CPmed (17.4 ± 0.3 g DM; $P < 0.05$). Structural and mineral components increased disproportionately with higher protein supply: chitin mass peaked at 5.2 ± 0.9 g DM and ash mass at 7.5 ± 0.3 g DM in CPhigh pupae, exceeding CPlow values by 33% and 25%, respectively. Overall, dietary protein level strongly influenced growth rate and nutrient partitioning, while the sequence of nutrient deposition remained conserved. These findings demonstrate that protein supply and harvest timing are key levers for optimising BSFL nutrient composition, performance and the targeted production of physiologically valuable nutrients in sustainable insect-based food and feed systems.

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Influence of diet composition on the NPK fertilising value of frass from *Tenebrio molitor*

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Frass generated during the rearing of *Tenebrio molitor* can be used as an organic fertiliser due to its NPK composition. As the main by-product of insect production, frass represents a key opportunity for valorisation within circular economy systems. However, variability in feed inputs used in yellow mealworm rearing leads to heterogeneity in the fertilising value of frass. This raises challenges for compliance with guaranteed nutrient regulations and for ensuring agronomic consistency. The objective of this study was to evaluate the impact of diet composition on the NPK fertilising value of frass and to model these relationships. A literature review revealed limited data directly linking diet composition to frass contents (n < 10). A feeding trial was therefore conducted at production scale, in which 10,000 larvae were reared for eleven weeks at 30 °C and 58% relative humidity under natural photoperiod. Ten dietary treatments were formulated from wheat bran, brewer's spent grain, okara, chicken feed, starch and yeast to generate a wide range of total dietary nitrogen (3.0–6.2%), phosphorus (0.3–1.9%) and potassium (0.1–2.2%) contents. Frass was harvested at the end of the trial and analysed for NPK content. Frass nutrient composition varied across diets, with total nitrogen (N) ranging from 2.0–4.2%, phosphorus (P_2O_5) from 1.0–6.0%, and potassium (K_2O) from 0.5–3.4%. Dietary nitrogen alone was not sufficient to explain variation of total nitrogen in frass. However, multiple linear regression integrating dietary N, P, and fibre was significant ($p < 0.01$; R^2 adj = 0.75). P_2O_5 and K_2O, contents of frass were explained by simple linear relationships with dietary phosphorus ($p < 0.001$; $R^2 = 0.86$) and potassium ($p < 0.001$; $R^2 = 0.93$), respectively. These findings demonstrate that dietary proximate composition directly influences the fertilising NPK values of frass and support the development of predictive models for frass nutrient composition. To date, this study is among the first to quantify these relationships under industrial-scale insect production conditions.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Optimising targeted mating in a black soldier fly breeding program

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Controlling parentage through targeted matings is a prerequisite for breeding programmes and genetic improvement. Indoor breeding of black soldier fly (BSF, *Hermetia illucens*) typically relies on unstructured mass-mating swarms, which provide limited control over individual mating events. Practical indoor protocols for efficient targeted matings remain non-trivial because mating behaviour and success depend on a plethora of environmental factors. We identified operational ranges for mating setups and environmental conditions that maximise the likelihood of successful crosses and developed a robust, repeatable targeted-mating protocol for indoor breeding of BSF. We conducted a series of iterative optimisation experiments using different small mating systems ("PP": 500 ml conical PP plastic cups (5 × 8 × 11 cm), "HD": PET boxes (9 × 9 × 5 cm), "GF": 900 ml PP boxes (11 × 8 × 10 cm), "net cage": 3.4 litre mosquito nets (15 × 15 × 15 cm)). We compared one-on-one pairings (female:male = 1:1) with polygynous setups with female-to-male ratios of 3:1, 4:1, 5:1, 8:1 and 10:1, and systematically evaluated key operational variables including illumination and husbandry factors such as adult feeding. Success was assessed using the proportion of females ovipositing fertile eggs under defined mating and oviposition conditions and the reproducibility of outcomes. Our results show that, across trials, polygynous setups proved more operationally reliable than one-on-one pairings. Oviposition was consistently highest at moderate female densities (4 – 5 females per male), where we regularly achieved 100% of females yielding fertile eggs. Occasional mating/oviposition failures highlighted sensitivity to microenvironmental factors, including differences in light intensity and duration, temperature, humidity and cage geometry, underscoring the need for standardised mating system setups and tightly controlled husbandry. Our study establishes a practical framework for repeatable targeted mating in BSF for breeding applications. The optimised protocol identifies a stable operating window, notably moderate female-to-male ratios, and emphasises controllable environmental drivers, especially illumination and microenvironment. This enables more dependable controlled crosses for breeding programmes.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Mating behaviour and sensory cues in BSF: key factors for optimising reproductive performance in mass rearing

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The black soldier fly (BSF), *Hermetia illucens*, is a key species of the circular economy due to its extraordinary larval bioconversion efficiency. While extensive research focuses on larval biomass production, a deeper understanding of adult reproductive biology is essential to further refine mass rearing protocols and ensure consistent performance in industrial facilities. This study provides a detailed ethogram of BSF mating behaviour, adds further information on BSF sexual dimorphism, and highlights sensory cues potentially involved in partner interactions and recognition. Morphological studies reveal marked sexual dimorphism in tarsal adhesive structures, with special discoidal setae, exclusively in males, suggesting their importance during mating. Behavioural observations, conducted through slow-motion video recordings, confirmed that the mating sequence is initiated by the female's flight, which immediately triggers the male's pursuit. A peculiar behaviour, "male hindlegs crossing," was identified for the first time: the male firmly grasps the female mid-air by crossing his hindlegs across the abdomen. After landing, the receptive female remains still while only the male performs vigorous wing fanning. The male simultaneously performs over the female: "tapping" behaviour, a rhythmic touch on the female's abdomen with the middle and hind legs, and "rubbing," where the male's forelegs are used over specific areas of the female's body. Likewise in Drosophila, these actions may serve as a form of communication between partners, involving (1) tactile stimulation of the female, (2) chemical perception through tarsal gustatory sensilla of cuticular hydrocarbons to assess sex recognition. Wing fanning may represent a signal of mating motivation for females or intraspecific competition with other males. Behavioural observations indicate that agonistic encounters between males exhibit interaction patterns that mirror these courtship attempts, suggesting a possible role in competition. In conclusion, this research offers practical insights into the reproductive biology of BSF, providing a scientific basis for the development of more efficient management strategies for adult populations in mass rearing systems.

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Patience pays: a successful multi-year breeding program in black soldier flies

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In 2019, a genetic improvement program was initiated to increase larval body weight in the black soldier fly (*Hermetia illucens*). Larval body weight increased consistently over 44 generations of directional selection (cumulative 133% gain in individual larval weight) with no indication of a phenotypic plateau, demonstrating potential for further genetic gains. Under automated production conditions, the selected line achieved a +34% increase in wet crate yield, +26% in dry matter yield, +32% in crude protein yield, and +21% in crude fat yield per crate relative to the base population. Throughout the selection program, key fitness components and production-relevant traits were monitored to identify potential correlated responses. There were no reproductive trade-offs associated with increased female body weight. Instead, the selected line showed an 18–49% increase in egg clutch weight per female, a 24–30% increase in the number of eggs per clutch, and a 3–4% increase in egg length. At the adult stage, sexual size dimorphism increased with overall body size, driven by a proportionally greater size increase in females than in males. Seven generations of selection-relaxation resulted in a 2% decline in body weight per generation, indicating that the genetic gains largely remain during several generations of no selection. Periodic refreshment with the selection line is recommended for long-term sustenance of benefits. Overall, this long-term breeding program demonstrates the power of artificial selection to improve biological performance and production efficiency in industrial Black Soldier Fly farming.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Sperm storage in BSF females reveals sexual conflicts: partial storage and digestion

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BSF is not only of great economic interest, but females have highly compartimentalized spermathecae, making it a valuable model for sexual selection investigations. Spatiotemporal dynamics of sperm storage after mating were described with fluorescence and transmission electron microscopy (TEM). Spermatozoa were counted both during and after mating in two successive spermathecae compartments: fishnet canals and reservoirs. In addition to seminal fluids that induce egg-laying and reduce female sexual receptivity, male transfers a sperm plug in the fishnet canals, then only some spermatozoa reach the reservoirs along 2 days. TEM observations of the fishnet canals revealed cells that may have digestive functions, explaining the decline in the number and viability of spermatozoa that have been not stored in the reservoirs. After one mating, females laid up to three fertile clutches, evidencing no constraints on sperm quantity or quality. Both spermatic and ultrastructural investigations strongly suggests that the ejaculate in BSF acts as a sperm plug, which is confirmed by paternity analyses. These results offer valuable perspectives to better understand male/female interactions during and after mating.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Metabolic regulatory networks for targeted nutrient synthesis in black soldier fly

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The black soldier fly (BSF) is a pivotal species for converting organic waste into high-value protein and lipids. However, the industrial application of BSF is currently constrained by unstable nutritional quality and an incomplete understanding of the metabolic mechanisms governing nutrient accumulation. To address the challenge of achieving precise, stable, and predictable production, this study aims to elucidate the key metabolic pathways and regulatory networks controlling lipid and protein synthesis in BSF throughout its life cycle. We constructed a genome-scale metabolic model (*iHil3966*) integrating life-cycle multi-omics. Dynamic proteomics and WGCNA mapped core larval regulatory networks. Key genes (FAS, ACC, FABP) and transcription factors (IVY, YBOX) were validated using RNAi, EMSA and dual-luciferase assays. Finally, model predictions were verified via nutritional intervention experiments with varying Carbohydrate: Protein ratios. Results confirm BSF nutritional plasticity. A diet with P+C = 70% (P:C 1:2.5) maximized survival (>95%) and biomass. The *iHil3966* model successfully predicted that supplementing low-value substrates with valine and isoleucine enhances biomass and fatty acid production. Molecularly, FAS, ACC, ACSBG and DGAT1 were identified as key lipid accumulation genes. RNAi silencing of HiACC and HiFAS significantly reduced triglycerides. We uncovered a mechanism where transcription factors IVY and YBOX negatively regulate lipid synthesis by binding *ACC* and *DGK* promoters. Additionally, FABP was identified as a central metabolic regulator downstream of *Eip75B*. Phosphoproteomics revealed that the TOR pathway (via IRS1 and PDK1) activates rapidly during the egg-to-larva transition. Crucially, we verified a metabolic trade-off where promoting fatty acid oxidation facilitates protein synthesis, providing a mechanism for targeted product quality regulation. This study establishes the first comprehensive multi-omics regulatory map of BSF growth and metabolism. By identifying core regulatory factors (such as FABP, IVY, and YBOX) and optimizing nutritional algorithms, we provide a robust theoretical framework for the "targeted synthesis" of insect nutrients. These findings offer scalable strategies for the insect industry to produce standardized, functional BSF products tailored for specific animal nutrition needs.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Identification of stable reference genes for RT-qPCR normalization in *Acheta domesticus*

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Acheta domesticus is attracting increasing interest as a sustainable protein source; however, molecular resources available for this species remain limited. The lack of validated reference genes constrains the reliability of RT-qPCR–based gene expression analyses, which are essential for investigating physiological, immune, and metabolic mechanisms in this insect. To identify and validate stable reference genes for RT-qPCR normalization in *A. domesticus*, taking into account different tissue types. The expression stability of six candidate reference genes (*AdoNEOPT*, *EF2*, *18S rRNA*, *EF*1α, *His H3* and *GAPDH*) was evaluated across five tissue types (abdomen, legs, wings, head, and whole body). Stability assessment was performed using multiple complementary approaches, including *BestKeeper*, *geNorm*, *NormFinder*, the ΔCt method and the integrated *RefFinder* platform. Statistical validation using R confirmed the robustness of the results. *EF*1α, *AdoNEOPT*, EF2 and 18S rRNA exhibited high expression stability across all analysed tissues. By contrast, *GAPDH* and *His H3* showed substantial variability and are generally not recommended as reference genes, with the exception of head tissue, where *GAPDH* displayed stable expression. This study represents the first validation of reference genes in *A. domesticus* and establishes a robust methodological foundation for accurate, reliable, and reproducible gene expression analyses. It paves the way for future research in functional genomics and for the development of novel qPCR-based diagnostic tools relying on molecular signatures. These advances will support health monitoring in insect farming systems, which remain vulnerable to emerging diseases, and will contribute to the development of more efficient and sustainable insect-based protein production systems.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Investigation and mitigation of a vertically transmissible pathogen in a black soldier fly breeding facility

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Biosecurity and disease management are major concerns for black soldier fly (BSF) farming operations. The farm investigated in this study exhibited severe larval mortality at the fifth and sixth instar stages (14–18 days old, coinciding with the onset of the prepupal stage), characterised by soft rot, orange-brown colouration, bloating, cessation of feeding activity, and a sludgy, foul-smelling substrate. This study investigated two aspects: (1) verification of suspected biological vertical transmission to enable targeted mitigation, and (2) evaluation of microbial dynamics of healthy and larvae with mortality Verification of vertical transmission was conducted using an inoculant prepared from residual substrate mixed with dead larvae collected from high-mortality trays. Experimental treatments were categorised by feedstock type, inoculum dosage (residual substrate, five dead larvae, or 2% substrate inclusion), microbial suppression strategy (potassium sorbate addition, pH reduction to pH 4, or inoculant sterilisation), tray hygiene (washed and sanitised, sanitised only, or unwashed), larval strain, and rearing environment. All treatments were initiated with 5000 four-day-old larvae and 500 g feedstock, and were refed ad libitum until mortality occurred or pupation was reached. Vertical transmission was confirmed, with faster onset and higher mortality at increased inoculation levels. Inoculation with residual substrate or as few as five dead larvae resulted in >90% mortality by day 10 post-setup. Sterilised inoculant treatments showed no mortality and complete pupation, whereas unsterilised treatments reached 100% mortality within 10 days. Tray hygiene trials demonstrated that washing or sanitising alone was insufficient to disrupt transmission; only the combined washing and sanitising protocol fully prevented mortality. Sanitisation using a disinfectant containing benzalkonium chloride and glutaraldehyde at 1% (v/v) was successfully implemented as mitigation. For microbial analysis, seedling larvae, healthy controls, and mortality-affected larvae were analysed using 16S rRNA gene sequencing. Distinct bacterial community profiles were observed between groups, although no dominant pathogenic taxa were identified. Low-abundance opportunistic bacteria, including *Wohlfahrtiimonas*, *Klebsiella*, and anaerobic taxa such as Desulfovibrio spp., were detected. Overall, this study verified the presence of vertically transmissible biological contamination at low inoculation thresholds and identified an effective sanitation-based mitigation measure. The findings highlight the importance of strict tray hygiene, routine colony health monitoring, and further research on BSF-specific pathogens

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Flies to feed the flock: a relative welfare exploration

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Welfare is a topic that gains rapid interest in research on insects as livestock feed. Yet, how this should be assessed remains a topic of exploration. Here we will address the behaviour of an insect as well as that of its predator, a bird, in the context of their behaviour. Video recordings have been made of the behaviour of black soldier fly (BSF) larvae to investigate their aggregation behaviour in dark conditions. Video recordings of the behaviour of poultry (broilers and layers) have been made when provided with BSF larvae, either as live larvae or as larval products. BSF larvae use cues related to conspecifics to aggregate and this behaviour is context dependent. We studied whether pathogen infection influenced this behaviour. Feeding live BSF larvae affected the behaviour of broilers and layers much more than the inclusion of BSF meal in the diet. Black solder fly larvae have adapted to exploit ephemeral food sources. They have evolved to rapidly develop on a diversity of resources. During development they are exposed to a diversity of threats including diseases and predation. This has resulted in behaviour that maximises the use of these resources while minimising the risks. The larvae exhibit dynamic aggregation behaviour which is mediated by cues from conspecifics. Is this information relevant for considerations of BSF welfare? The vast majority of birds on our planet use insects as food source. Wild relatives of poultry birds have evolved as omnivores that readily include insects in their diet and actively forage to find such prey. Insects provide an important source of proteins for adults as well as their chicks. When provided with BSF larvae broilers as well as laying hens readily eat them and this influences their overall behaviour. However, the vast majority of poultry birds do not have access to insects as a food source. Do BSF larvae contribute to poultry welfare? The final element of this presentation will be how to integrate welfare considerations of BSF and poultry when producing animal proteins to feed humans.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Optimising mealworm welfare: results from feeding and environmental trials at Bobo Eco Farm

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Optimising rearing practices is essential for improving efficiency and productivity in mealworm production. This study investigates the impact of diet, environmental conditions, and management strategies on mealworm production. Experiments focused on feeding strategies for egg hatching, larvae development, adult welfare, pupa survival, and post-split larvae feeding. Alternative substrates, such as wheat bran, cassava peels, wet feeding, and protein supplementation with black soldier fly larvae (BSFL), were also tested. At Bobo Eco Farm, larvae were reared in trays with a 50:50 mixture of maize bran (MB) and crushed maize cobs (MC). 100g of split larvae were placed in each tray with 1000 g of substrate. Refeeding and frassing were done biweekly. Trials evaluated diets (MB, wheat bran (WB), and MB + MC) on larvae development and egg hatchability over 6–14 weeks. Adult welfare was assessed in warmer (26–31 °C, 55–70% humidity) and cooler (22–25 °C, 65–80% humidity) rooms. Pupa survival and feeding strategies, including one-time and continuous refeeding, were tested. Wet feeding and alternative substrates were also studied. Larvae fed MB reached split in 8 weeks, while MB + MC took 11 weeks. MC-fed larvae split at 14 weeks. Wheat bran-fed larvae split in 6 weeks, while MB larvae took 9 weeks. WB larvae were larger and easier to separate from frass. Frass as a substrate increased egg yield. Cooler conditions improved adult survival and egg production. Pupa survival dropped from 60% to 30% in warmer conditions. Wet feeding accelerated larvae growth, with wet-fed larvae reaching split in 7–8 weeks, compared to 11–12 weeks for dry-fed larvae. BSFL-fed larvae split in 8 weeks, while wheat bran-fed larvae took 13 weeks. This study highlights the importance of improving welfare conditions to enhance mealworm rearing efficiency. Providing optimal diets, such as WB over MB, accelerated larvae development and minimised stress. Lower temperatures and higher humidity improved adult survival and egg production. Welfare improvements through diet, environmental management, and feeding practices result in healthier and more productive mealworms. Optimising mealworm welfare through diet, environmental conditions, and wet feeding significantly enhanced productivity. By focusing on welfare, mealworms exhibited faster growth, higher survival, and better reproductive success, essential for sustainable mealworm farming.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Genetic effects on larval performance and bioconversion efficiency in *Hermetia illucens*: validation across generations

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The black soldier fly (*Hermetia illucens*) is widely studied for its ability to convert low-value organic substrates into high-value insect biomass. In practice, these substrates serve as larval diets and can vary strongly in nutrient composition, fibre content and digestibility, which has a major impact on larval growth, yield and conversion efficiency. While substrate effects on performance are becoming well documented, far less is known about how genetic differences between *H. illucens* strains interact with nutritionally contrasting diets, and whether such genotype-by-environment (G × E) effects are stable across time. In this study, we evaluated the influence of genetic background on larval performance and bioconversion efficiency on different substrates across time by repeatedly assessing nine genetically distinct captive *H. illucens* strains, representing European lineages (4 populations), African lineages (2 populations) and Oceanian lineages (3 populations). Strains were genotyped using microsatellites markers and whole-genome sequencing and reared under standardized conditions over the course of 10 generations during this study. Larval performance was assessed on three nutritionally contrasting diets: a nutrient-rich chicken start mash, a cereal-based wheat bran, and a high-fibre horse feed. Following an extensive initial feeding trial, the experiment was repeated in two additional trials conducted over non-consecutive generations. Across all trials, significant effects of strain and substrate were observed for larval yield and bioconversion efficiency. While absolute performance values differed between trials, normalization revealed that relative strain performance remained largely consistent over time. This study demonstrates the importance of genetic background for bioconversion efficiency.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Exploring the hemolymph microbiome: an emerging lever for health management in industrial *Tenebrio molitor* rearing

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The farming of edible insects represents a promising alternative strategy for the sustainable production of protein-rich food and feed with a low environmental footprint. Rearing substrates strongly influence insect performance and play a key role in shaping insect- associated microbiota, which in turn can affect host physiology and immune competence. While gut microbiota has been extensively investigated, the microbiota associated with insect hemolymph and its potential role in immune responses remain largely unexplored. The HeMiTool project (Hemolymph Microbiome of insects: a promising Tool to develop innovative strategies to control pests and protect beneficials) aims to fill this knowledge gap by providing an in-depth characterisation of hemolymph microbiome–immune system dynamics in mature larvae of *Tenebrio molitor* L. Larvae were continuously reared on different agro-industrial by-products under controlled conditions, allowing a robust comparison of diet-related effects. Hemolymph and gut microbial communities were analysed using a combined culture- dependent and culture-independent approach, including high-throughput 16S rRNA gene sequencing and quantitative PCR. In parallel, key chemical and biochemical parameters of the hemolymph were assessed, and some immune-related traits were investigated. Methodological optimisation of hemolymph extraction and processing ensured the reliability of downstream microbiological and immunological analyses. Preliminary results indicate that both hemolymph and gut-associated microbial communities are influenced by the rearing substrate, supporting the existence of a previously overlooked, diet-responsive hemolymph microbiome compartment. Observed trends in hemolymph- associated parameters suggest possible interactions between nutrition and host physiological status, although further investigation is required to clarify these relationships. Overall, this study provides first insights into the hemolymph microbiome of an edible insect species and highlights its relevance as an emerging component of insect biology. These findings provide a foundation for future diet-based strategies aimed at improving robustness and health stability in mass-reared edible insects. This work was funded by the PRIN 2022 PNRR project HeMiTool (Grant no. P20228WWB7), European Union – Next Generation EU, Mission 4 Component 1.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Black soldier fly (*Hermetia illucens*) characterisation of gut microbiome

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The exact role and functions of the microbiome of black soldier fly (*Hermetia illucens*) (BSFL) are not known, however it is essential for them to convert organic waste into high-value biomass, while enhancing said waste into protein and nutrients suitable for other animal consumption. It is important to identify the microbes of the gut in order to characterise possible probiotics or pre-fermentation advantages as both qualitative and quantifying microbial communities informing possible production activities that will maximise BSFL production outcomes. This study examines the taxonomic structure and abundancy of the intestinal bacterial communities of BSFL while fed on selective organic waste, based on the bacterial communities within the full length of BSFL gastrointestinal tract. In addition, the bacterial profiling of the waste substrates samples provides a catalogue of the environmental bacterial taxa. Bacterial assemblages were evaluated using Illumina deep-sequencing of the hypervariable region (V1-V2) of the 16S rRNA gene amplified from each sample. 738 bacteria were identified in 70 samples of larvae and 18 samples of frass, characterising the top phyla among all the communities in terms of relative abundance as Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria. Firmicutes, Actinobacteria and Bacteroidetes were the dominant phyla in the BSFL gut of all organic waste systems with egg substrate BSFL showing additional abundance in Proteobacteria, while Firmicutes dominate the frass, with the addition of Actinobacteria and Pseudomonadales in the egg and mixed substrates. In frass samples the presence of synthesising amino acids, Rhizobiales, Burkholderia and Bacteroidales were found however they were not present in any larvae samples. Key analysis based on genes showed that intestinal microbes' communities within BSFL differ to their respective frass microbe abundance. These results clarify the biological mechanisms of high-efficiency nutrient conversion in BSFL associated with microbes.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Enhancing insect production with innovative microbial solutions

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The rapid growth of the insect farming industry presents both opportunities and challenges for producers seeking to optimise productivity and sustainability. A key factor in ensuring the success of insect rearing operations is the ability to maintain optimal gut health and nutrient utilisation in the black soldier fly larvae. In this presentation, we will share Phileo by Lesaffre latest findings from our research and industrial trials on the application of innovative microbial solutions to enhance insect performance, create functional products, and reduce GHG emissions. Our trials have demonstrated that the strategic incorporation of probiotics and postbiotics into insect diets can significantly improve feed conversion ratios, increase larval weight gain, and reduce developmental time. By leveraging the natural synergies between insects and microbes, our solutions help to stabilize substrate and intestinal environment and bolster the insect's immune response. This, in turn, leads to increased productivity, improved product quality and enhanced sustainability for insect farming operations. Drawing on industrial scale studies and data-driven insights, we will explore the mechanisms by which our microbial additives deliver these benefits, as well as provide guidance on the practical implementation of these technologies within commercial insect production systems. Attendees will gain a comprehensive understanding of how innovative microbial solutions can unlock the full potential of insect farming and position the industry for long-term success.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Influence of protein-enriched diets on the larval development of *Tenebrio molitor*

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Tenebrio molitor (Tm) is a sustainable protein source, and optimising mass rearing requires valorising agri-food byproducts (Sangiorgio *et al.*, 2022). Wheat bran is the standard feed, but balanced macronutrients are essential for profitable yield (Kröncke & Benning, 2022), as high dietary protein alone, in addition to be costly, does not ensure growth when digestibility is low (Montalbán *et al.*, 2022). This study assessed three protein-rich byproducts on Tm larval growth and protein assimilation. Eggs from one cohort were reared on wheat bran until week 6, then assigned to seven dietary treatments (T): 100% wheat bran (control) or wheat bran partially replaced (40 or 30%) with byproduct A (T1–2), B (T3–4), or C (T5–6), yielding approx. 18–23% dietary protein. Five replicates per treatment were maintained at 27 °C and 70% RH with *ad libitum* feeding. Larval mass was measured weekly (20 larvae/replicate, randomly sampled with replacement) until pupation. Larval and frass crude protein were analysed using the Kjeldahl method (Yu *et al.*, 2021). A Gamma GLMM revealed a strong effect of time on larval mass ($\chi^2(7) = 6876.1$, $p < 0.0001$) and a highly significant treatment × week interaction ($\chi^2(42) = 775.5$, $p < 0.0001$), while initial treatment effects were absent. This demonstrates that treatment effects emerged as larvae grew. Estimated marginal means indicated that differences appeared in week 7, and were clearer in week 8, where T3–T4 consistently formed the lowest-mass groups. By weeks 10–13, treatments formed three to four distinct statistical clusters per week, with stable rankings: the control and T1–2 had the highest masses, while T3–5 remained the lowest. For the assessment of protein assimilation, preliminary analysis shows higher nitrogen use efficiency in experimental diets, with protein assimilation reaching approx. 20% and feed–protein conversion increasing to 8.5–8.8% versus 7.1% in the control, while body protein content remained stable (approx. 46%). Thus, dietary effects reflected improved protein use rather than altered body protein content. The results demonstrate that clear divergences in growth performance emerge in later instars. Control and byproduct A (T1–T2) showed the highest masses, confirming wheat bran's efficacy (Bordiean *et al.*, 2022). The poor performance of byproduct B (T3–T4) suggests nutritional imbalance (Morales-Ramos *et al.*, 2013) or lower digestibility (Zhang *et al.*, 2019). This highlights that the entire nutritional matrix, not just protein quantity, must be evaluated for effective diet formulation.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Revealing the reproductive biology of the black soldier fly: from age determination to mating dynamics

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The black soldier fly (BSF, *Hermetia illucens*) is a key species for sustainable bioconversion and production of protein, oil and organic fertilisers. Despite its industrial importance, many aspects of its reproductive biology remain poorly understood. To support optimization of mass-rearing systems, a series of studies were conducted to clarify key factors regulating reproductive performance in BSF. Age determination, reproductive morphology and mating performance were examined under controlled environmental conditions. A total of 2500 adults were analysed to identify morphological and physiological age markers, including abdominal "windows," ovarian development, and mesothoracic phragmata. For reproductive analysis, 700 females were dissected and classified into five ovarian developmental stages. Ovarian length (L), width (W) and index (L/W) were measured, together with wing length (LW) and thorax length (TL) as maturity indicators. Oviposition was studied using 120 egg clutches observed for 5 days to assess fertility, total egg number and the proportion of infertile eggs. To evaluate the influence of body size on reproduction, 720 adults were grouped into four sex – size combinations (large – large, small – small, small male – large female, large male – small female) with a 1:1 sex ratio and 60 flies per cage. Data was analysed using IBM SPSS Statistics. Distinct changes in abdominal "windows," ovarian morphology, and mesothoracic phragmata correlated strongly with chronological age ($p < 0.001$), enabling accurate identification of sexual maturity and synchronization of breeding cohorts. Ovarian morphometry revealed five developmental stages; L and OI were reliable indicators of reproductive status, while body size alone was insufficient. Infertile eggs (6 – 16% per clutch) appeared consistently and correlated with clutch size ($r = 0.97$, $p < 0.001$), possibly indicating a physiological or behavioral function. Reproductive success depended mainly on the size ratio between males and females, not absolute size. The highest mating success (88 – 96%) occurred when the female:male TL ratio ranged from 0.95 to 1.12 ($R^2 = 0.91$, $p < 0.001$). Small males showed greater agility, while large females produced more eggs, suggesting that optimal size complementarity maximises mating efficiency and reproductive output. Collectively, these findings deepen understanding of BSF reproductive performance by linking morphological age markers, ovarian maturation, mating dynamics and reproductive output. The integration of these parameters provides practical tools for assessing adult readiness, synchronising breeding populations, and enhancing egg production efficiency in industrial-scale BSF rearing.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

How larval diet composition and switching shape adult fitness in the black soldier fly

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Larval nutrition strongly shapes adult life-history traits and reproductive output in insects. However, most experimental studies use a single diet throughout larval development, an approach that poorly reflects the nutritionally variable conditions insects experience in nature. Understanding how insects respond to such nutritional variability is essential for improving production efficiency under real-world conditions. Here, we examined how larval diet composition and dietary switching influence adult fitness traits in the black soldier fly (*Hermetia illucens*), a species whose adults do not feed and rely entirely on nutrients accumulated during larval development. Larvae were reared on either a nutritionally complex, balanced diet (Gainesville diet) or a nutritionally simple monodiet (corn). Using a full-factorial design, we tested eight larval diet regimes in which individuals either remained on one diet (low-quality (LLL) or high-quality (HHH)) or experienced one ((HHL, HLL, LHH, LLH)) or two ((HLH, LHL)) switches in diet quality. We measured larval growth rate and assessed adult body size, fecundity, sexual dimorphism, longevity, and reproductive performance. We predict strong, trait-specific plasticity in response to larval nutrition, with individuals reared predominantly on the complex diet exhibiting higher fitness than those reared on the monodiet. We also expect diet variability to produce non-linear outcomes driven by ontogeny-specific sensitivity to nutrition. Overall, our study provides insights into how variable larval nutrition influences performance-relevant traits in *H. illucens*, with direct implications for optimising feeding strategies, breeding programs, and production stability in insect food and feed systems.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Microbial partners in immunity: probiotic effects on yellow mealworm and black soldier fly physiology

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In these years, the identification of potential probiotic microorganisms within the microbiome of farmed insects, as well as their application in mass-rearing systems, has been increasingly investigated to evaluate their effects on insect growth, reproduction and overall health with the additional aim of mitigating multiple stressors such as entomopathogens, temperature fluctuations and high rearing densities. The PRIN 2022 Project InProFarm (Insect Probiotic-assisted Farming: a promising tool to enhance edible insect health and performances) explored the interactions between beneficial bacterial symbionts and two mass-reared species, *Tenebrio molitor* (yellow mealworm) and *Hermetia illucens* (black soldier fly), and their impact on humoral and cellular immune responses. Beneficial bacterial symbionts were screened from larvae of both species, and their probiotic potential was assessed through *in vitro* and *in vivo* assays, including feed administration, evaluation of insect performance, and analysis of microbiota composition. In addition to growth performance, genes involved in growth and immune pathways were analysed to uncover the effects of probiotic supplementation on key traits relevant to insects breeding, such as antimicrobial peptides production and reproduction. Overall, the study highlights the importance of promoting healthy and safe insect production systems for feed and food applications. This work was funded by the PRIN 2022 Project InProFarm (Insect Probiotic-assisted Farming: a promising tool to enhance edible insect health and performances), grant number 2022L4NJMK, funded by the European Union — Next Generation EU, Mission 4 Component 1.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Repeat-rich and rapidly evolving: the TE landscape of the black soldier fly

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Transposable elements (TEs) are major contributors to genome structure, regulation, and evolutionary change in insects. As *Hermetia illucens* (BSF) becomes central to global insect agriculture, clarifying its TE landscape is critical for understanding genomic plasticity, domestication processes, and phenotypic variability is relevant to industrial production. We examined whole-genome repeat composition in BSF using curated TE annotations and divergence profiles to characterise TE classes, relative abundance, and signatures of historical versus recent TE activity in four *de novo* genomes. BSF displays an exceptionally high repeat content (67.6–70.8%), positioning it among the most TE dense insect genomes and reflecting a genomic architecture more complex than traditional insect species. The TE community is composed of substantial LINE, DNA transposon, and unclassified repeat fractions, with evidence for both ancient TE expansions and more recent proliferation events. Class-level TE diversity was nearly identical among genomes, but multiple DNA transposon families showed distinct lineage-specific differences. Patterns of TE diversity may suggest robust mechanisms of genomic variability and provide a framework for understanding lineage specific differences that may influence traits such as development rate, stress tolerance, and reproductive performance. TE driven genomic flexibility likely contributes to the phenotypic variation observed across commercial and laboratory strains, which may make TE associated markers valuable tools for selective breeding and population management. These results highlight the importance of incorporating TE dynamics into ongoing genomic, evolutionary, and applied research efforts. By defining the TE architecture of *H. illucens*, this study provides foundational genomic insight for a species rapidly becoming indispensable to sustainable insect agriculture.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Water requirements and their effects on growth and gene expression in the yellow mealworm

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Numerous studies have shown that insect growth depends on adequate water supply, including in the yellow mealworm (*Tenebrio molitor* L.). Although Y. mealworm can develop on dry feed, supplementary water in industrial rearing can accelerate its growth. However, data on the effects of water supply levels and sources remain limited. Therefore, our research team has been conducting studies in this field in recent years. Our preliminary studies indicate that water deficiency significantly reduces the growth rate and biomass production of mealworm larvae, while inducing defence responses manifested by increased expression of water-stress-related genes, such as HSP70 and Coleoptericin B. In addition, analysis of P450 gene expression proved to be a useful indicator for assessing moderate water deficit in insects. Moreover, both the amount of water supplied to the insects and its source affect their growth and development, and larvae without access to an additional water source can attain up to a fourfold lower body mass than larvae provided with an additional source of water. The lack of supplementary water, however, does not significantly affect larval survival, which remains high at approx. 90–98%. When comparing water sources, larvae supplied with carrot as a water source achieved a slightly higher body mass (128 mg per individual) than those provided with agar gel (114 mg per individual), although it should be noted that this difference was not statistically significant. In conclusion, the water source and its rate can significantly affect the development of mealworm larvae in industrial rearing. Therefore, determining the optimal rate of supplementary water and its source is of key importance, as this can substantially influence the profitability of insect production as well as its environmental impact (e.g. carbon or water footprint). For this reason, our research team will continue investigations in this field. This research was funded by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, through the Department of Genetics, Plant Breeding, and Bioresource Engineering (topic number 30.610.007-110) and the Department of Entomology, Phytopathology, and Molecular Diagnostics (topic number 30.610.011-110). Moreover, it was funded by the Minister of Science under "the Regional Initiative of Excellence Program."

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Digestibility of cereal-based nutritional substrates in *Tenebrio molitor*

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In the context of sustainable insect production and circular bioeconomy, increasing attention has been given to the use of agricultural residues and agro-industrial byproducts as alternative substrates for rearing *Tenebrio molitor* larvae. *Tenebrio molitor* exhibits a high capacity to adapt to diverse substrates, which is associated with its ability to degrade complex carbohydrates such as cellulose. However, the specific digestive efficiency and metabolic responses of this species to low-quality feed remain insufficiently understood, highlighting the importance of further research on food digestibility in this insect. Some of the production parameters and digestibility were evaluated during the rearing of *T. molitor*. The experiment was initiated with 25 g of 30-day-old *Tenebrio molitor* larvae and lasted for six weeks, during which weekly weight gain, feed intake, frass production, and feed conversion were recorded. For the experiment it was used eight different feedstuffs. All parameters were measured per tray. In terms of total weight gain for the whole period, the highest gain was observed in the group fed with wheat bran (average 39.97 g/tray), while the lowest weight gain was observed in the group fed with breadcrumbs (only 9.85 g/tray). The digestibility coefficient for each feed was calculated based on the feed consumption and the frass production. The highest digestibility was observed in oat flakes (0.78) and the lowest in sunflower meal (0.30). While the weight gain was very low in the group with breadcrumbs, the digestibility was high (average 0.77). The feed conversion ratios for wheat bran, sunflower meal, breadcrumbs, oat, oat flakes, barley, barley flakes, and DDGs were 2.11, 3.8, 8.6, 4.02, 3.49, 7.05, 3.80 and 1.95, respectively. Overall, *T. molitor* larvae showed the best growth performance and feed utilisation when reared on wheat bran, as indicated by the highest total weight gain and the lowest feed conversion index compared with other used feed. These results confirm the strong adaptability of this species to different cereal-based substrates and underline the importance of substrate quality for optimising digestibility and production efficiency in sustainable insect rearing systems.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Safeguarding insect mass-rearing: molecular approaches to pathogen identification and surveillance

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Insect mass-rearing is a rapidly growing field with applications ranging from sustainable protein production to biocontrol. While most research has focused on improving production efficiency, the health of reared insects has received comparatively less attention, despite its critical impact on industry sustainability. Our work aims to bridge this gap by applying advanced scientific methods to detect and monitor pathogens threatening insect-rearing operations. By combining specialized databases of insect pathogens with high-throughput sequencing, we have identified novel pathogens associated with impaired production in mass-reared insects, such as the black soldier fly, the mealworm, and biocontrol agents of the *Nesidiocoris* genus. In parallel, we have developed targeted molecular techniques to deliver cost-effective and tailored approaches for the detection and quantification of known insect pathogens. Overall, these case studies highlight the importance of integrating pathogen discovery and health monitoring into standard rearing practices to ensure the resilience and safety of this emerging industry.

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Assessment of physiological status and antimicrobial potential in larvae of *H. illucens* reared on aquaculture sludge

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The bioconversion of aquaculture sludge by *Hermetia illucens* larvae (BSFL) represents a key strategy for the circular economy. It is well established that the nature of the growth substrate can modulate the insect's physiology and immune response. However, the energetic cost of such adaptation remains less explored. The present study aims to verify the substrate-induced antibacterial properties of the hemolymph against *Aeromonas salmonicida*, a major fish pathogen, and to evaluate the temporal dynamics of the stress response (Heat Shock Protein — HSP70) and metabolic vigor (Vitellogenin — VTG). Fourth instar larvae were subjected to three different diets: Control (Gainesville diet), 50% sludge + 50% Gainesville diet, and 100% aquaculture sludge. Sampling was performed at T0 (baseline), T1 (4 hours), and T2 (24 hours). Gene expression analysis for VTG and HSP70 was conducted via qPCR on cDNA, normalizing data against the 16S gene. Concurrently, hemolymph bactericidal activity was evaluated using *in vitro* antibiogram assays. Molecular tests showed at T1 (4h) a significant up-regulation of VTG in the 100% sludge group (Fold Change 3.38). At T2 (24h), VTG expression underwent a marked reduction (FC 0.07 in the 50% sludge group). HSP70 expression showed a significant increase exclusively at 24h in the 100% group (FC 3.36), suggesting the late activation of cellular protection mechanisms. Microbiological analyses against *A. salmonicida* revealed distinct patterns: in the Control group (T1 and T2), hemolymph produced an inhibition zone of approx. 6.6 mm with the presence of internal colonies. The 50% sludge group generated a wider zone (6.9 mm), albeit with colonies present within. In the 100% sludge group, despite a reduced zone (approx. 5.8 mm), a total absence of colonies within the zone was observed. Data integration highlights an energetic trade-off. Although defensive efficacy remains maximal in the 100% sludge group, VTG analysis reveals the sustained metabolic effort: the initial overexpression (T1) suggests energetic mobilization, while the subsequent drop (T2) indicates potential transcriptional exhaustion, with the larva downregulating growth functions to prioritize survival. The delayed activation of HSP70 suggests the intervention of chaperones to counteract proteotoxic damage. In conclusion, pure sludge acts as a potent immunostimulant but imposes a significant physiological cost, as evidenced by the molecular pattern.

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Potential host-parasite relationships between the parasitoid hymenoptera *Trichopria drosophilae* and *Hermetia illucens*

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Hermetia illucens (Linnaeus), the black soldier fly (BSF), is undoubtedly the most studied species among those recently authorised by European regulations for use in insect meal as a protein source in feed for poultry, swine, and fish. However, despite the extensive volume of studies available on this species, knowledge regarding its potential pathogens remains scarce. An analysis of specific literature indicates that the pupal stages of the black soldier fly could be parasitized by certain species of the genus *Trichopria* (Hymenoptera, Diapriidae). The aim of the present study was to verify experimentally whether the parasitoid *Trichopria* could have an actual parasitic incidence on *H. illucens* rearing systems. This is particularly important considering that *T. drosophilae* is currently used for the biological control of *Drosophila suzukii* Matsumura, which was recently accidentally introduced into the country. *T. drosophilae* has been commercially available in Italy since 2017, is commonly on sale, and has been widely and regularly released across the national territory. The possible parasitic action exerted by the parasitoid *T. drosophilae* towards the pupal stages of *H. illucens* was considered, with the aim of potentially suggesting the implementation of containment measures in facilities intended for rearing the latter, ranging from large centralized plants to smaller, locally-based structures typical of circular economy models. To verify the type and degree of interspecific interactions, an experimental laboratory setup was created to house the larval stages of *H. illucens* and *D. melanogaster* together with adults of *T. drosophilae*. Three series of experiments were carried out by exposing *T. drosophilae* females, previously kept for 24 hours with males, to *D. melanogaster* pupae and the different larval stages of *H. illucens* for 48 hours under different experimental conditions. *T. drosophilae* was shown to parasitize *D. melanogaster* in all trials but never parasitized *H. illucens*. When placed with *D. melanogaster*, *Trichopria* exhibited parasitisation rates varying between 40% and 80%. *D. melanogaster* placed in the absence of *Trichopria* (negative control) showed very high success and emergence rates (75%). *H. illucens* in the absence of *Trichopria* (negative control) had equally high success rates (80%); when placed together, no larval stage or pupa was parasitized, and the emergence rate remained high.

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Abundance of selected amino acid transporter mRNAs in the gut of starved and fed black soldier fly larvae

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The growth of black soldier fly larvae (BSFL) depends on availability and uptake of nutrients such as amino acids (AA), which occurs via intestinal AA transporters (AAT). The capacity and affinity of these AAT are responsible for the efficiency of AA uptake, with their abundance depending on the insect's developmental status and feed supply. In this initial work, we quantified the expression of several putative AAT transcripts in the mid- and hindgut of fed and starved BSFL. The BSFL (12 vessels each with 150 larvae) were kept at 27.5 °C and 65% relative humidity until harvest on day (d) 16 and fed chicken feed (CF). On d 15, larvae from 6 vessels were isolated and kept separately without feeding substrate for 24 h (starved; n = 6) while larvae in remaining 6 vessels continued on CF until d 16 (fed; n = 6). At harvest, all larvae were isolated from the substrate, washed, dried and frozen in liquid nitrogen. The hindgut and the posterior midgut were isolated from 5 starved and 5 fed frozen larvae/vessel and analysed as pooled samples. After extraction, the reverse transcribed RNA was quantified by qPCR. Primers were selected based on predicted sequences (NIH GenBank). The data were analysed by PROC NPAR1WAY (SAS 9.4) with the Wilcoxon test (significance: $P < 0.05$, trend: $0.05<P < 0.1$). Four putative neutral AAT (XR_005249910.1 (N1), XM_038061550.1 (N2), XM_038062991 (N3), XM_038064401.1 (N4)) and two putative cationic AAT (XM_038052742.1 (C1), XM_038052605.1 (C2)) were quantified in mid- and hindgut of BSFL. In the midgut of starved larvae, N4 abundance was 1.8 times higher than in fed larvae ($P = 0.054$). In the hindgut, abundance of N2 was 4 times higher ($P = 0.017$) and N4 ($P = 0.093$) was 2 times higher in starved larvae, while C1 ($P = 0.055$) was 3 times higher in fed larvae. When comparing midgut vs. hindgut of starved larvae, N1 was 6 times higher in the hindgut ($P = 0.017$) and C2 was 13 times higher in the midgut ($P = 0.017$). In fed BSFL, N1 was 12 times higher in the hindgut ($P = 0.017$) and C2 was 3 times higher in the midgut ($P = 0.04$). All AATs analysed were expressed in midgut and hindgut of BSFL. Based on their abundance, the neutral AAT N1 and N2 and the cationic AAT C2 seem to be the most relevant in the current experimental setting. Judging by their abundance, C2 appears to respond primarily to starvation in midgut, while N1 and N2 in hindgut. The AAT N1 seems to be relevant for absorption of AA from the gut regardless of feeding status. The expression of N1 and N2 in the hindgut could be important for the recovery of AA from primary urine. In conclusion, under starvation, most of the AAT mRNAs analysed in the mid- and hindgut responded differently to differences in AA availability.

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Hybridisation among geographically distinct *Tenebrio molitor* strains: multigenerational effects on key biological traits

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The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), has been widely studied and mass-reared for decades, yet many aspects of its rearing optimisation remain insufficiently explored. Crossbreeding geographically distinct *T. molitor* strains offers a promising path for improving key biological traits, but this approach has not been thoroughly examined. This study investigated the effects of reciprocal hybridisation on reproductive performance, adult longevity, and larval growth across multiple generations, using four purebred *T. molitor* strains originating from Greece, Italy, Germany and the USA. Adult reproductive output and survival were monitored in both inbred and outbred lines by weekly recording of egg production, hatchability and adult survival in the F0 and F1 generations. Larval performance was assessed in the F3 generation by measuring larval weight and survival every 14 days until the appearance of the first pupae. Performance differed significantly among crosses for all traits across all generations. Several outbred lines showed clear reciprocal effects, while two hybrids, i.e., German (♀) × USA (♂) and USA (♀) × German (♂), exhibited heterotic effects in both fecundity and fertility. Adult survival also varied significantly among strains in both generations. Although the inbred Italian strain outperformed all other inbred and outbred crosses, the German (♀) × USA (♂) hybrid consistently showed the strongest performance across all life-history traits and generations among the outbred crosses. In the F3 generation, the Greek (♀) × German (♂) hybrid displayed the shortest development time (61 days). Overall, these results underscore the potential of targeted crossbreeding strategies to enhance *T. molitor* mass-rearing efficiency and support the development of strains better suited for industrial-scale production. Acknowledgement: This study was supported by project “Mealworm Innovations” (MIS: 7591), carried out within the framework of the National Recovery and Resilience Plan Greece 2.0, funded by the European Union – NextGenerationEU (Implementation body: HFRI).

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High-throughput computer vision pipeline to study mating behavior across 10 strains of black soldier fly

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The black soldier fly, *Hermetia illucens*, is mass-reared throughout the globe to valorise organic waste into many products including animal feed. For the success of industrial mass-rearing programs, efficient breeding is needed. While studies have begun to reveal a complex genomic landscape in wild and industrial BSF strains, practically nothing is known about how BSF strains of different genetic provenances may exhibit different reproductive phenotypes, nor potentially contribute to reproductive incompatibility or hybrid vigor. Of course, part of the reason is that behavioural assays of adult BSF are time consuming, labor intensive, and low throughput. Here, we present on a computer-vision apparatus and machine-learning pipeline that directly address this need; a retrofitted 30 x 30 x 30 cm cage automatically records images of live BSF breeding populations, after which a pipeline extracts meaningful features from these images (e.g., number of mating pairs, motion of males and females, etc.). Preliminary experimentation using the verified system will focus on detecting observable differences in mating behaviour and fitness during 6-day experiments (replicated n = 3 times) in response to shifts in cohort demography (i.e., ages), sex ratio, population density, and abiotic conditions. Thus, this system is well-poised to quickly document the BSF reproductive behavioural phenome, which will be informative for ongoing and future industrial breeding programs.

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Developing a mutagenesis breeding protocol for black soldier fly: evaluating UV, EMS, and colchicine on eggs

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The genetic improvement of the black soldier fly (BSF), a key resource insect for waste bioconversion, is constrained by limited germplasm diversity domestically. Introducing foreign strains is challenging. Mutagenesis breeding, proven in plants and Drosophila, offers a solution to create novel genetic variation. However, its efficacy in BSF, particularly at the egg stage, remains unexplored. This study systematically compared common mutagens to establish a foundational breeding protocol for BSF. Eggs from a laboratory population were treated with ultraviolet radiation (UVA/UVC at various durations), the alkylating agent EMS (0.5–2%), or colchicine (0.5–5%). A negative control was included. Treatments were applied consecutively over five generations (P1-P5). Hatching rates were recorded. Genetic divergence between treated and control groups was assessed by genotyping adults from the P5 generation using a custom-designed 40K SNP liquid chip and analysing genetic distances. Hatching rate analysis showed UVC caused the highest lethality, while colchicine had minimal effect. Genetic analysis revealed a critical divergence: UV treatments induced significant genetic differentiation from the control group after five generations. By contrast, EMS treatments, despite causing dose-dependent lethality, showed no significant genetic divergence from the control. Colchicine was ineffective and not pursued beyond initial tests. The results demonstrate that UV irradiation, not EMS, is effective for inducing heritable genetic variation in BSF eggs under our protocol. This suggests different mutagens have distinct effect modes in BSF–UV causes genomic changes viable for selection, while EMS's primary effect is physiological toxicity. We have successfully developed a practical mutagenesis workflow using UV treatment on eggs and high-density SNP chip verification. This protocol provides a powerful new tool to expand genetic diversity for BSF breeding programs, mitigating reliance on limited native germplasm.

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Does size matter? Allometry in adult black soldier flies under body-weight selection

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Directional selection for increased body size is expected to generate coordinated morphological responses through changes in cell size and number, potentially altering trait proportionality, sexual dimorphism and functional performance. Quantifying these allometric responses is therefore essential for predicting outcomes and trade-offs in long-term breeding programs. We investigated allometric patterns across 39 generations of artificial selection for increased body size in the black soldier fly (*Hermetia illucens*). Specifically, we tested whether trait enlargement is proportional, allometric relationships change over generations and differential responses between sexes. We analysed 331 adult flies from the parental populations of body-weight line under selection for generations 2, 20 and 39 using a combination of traditional linear morphometrics (on head width and tibia length) and landmark-based geometric morphometrics (on wings). Linear traits were evaluated relative to wing centroid size (CS), whereas wing shape variation was assessed following Generalized Procrustes Analysis and multivariate regression of shape on size. All three traits showed that sexual size dimorphism (SSD) increased with selected generations, with a proportionally greater size increase in females than in males. Strong proportional scaling was detected between log values of head width, tibia length and wing CS (r = 0.77–0.92), with comparable patterns in males and females, indicating largely isometric growth of major body components under selection. Larger adults exhibited proportionately larger wings, heads, and legs across all generations. Although wing shape varied significantly between sexes, the contribution of allometry to shape variation remained low throughout the selection program, ranging between approx. 1% and 3.3%. Overall, our results demonstrate that long-term selection for increased body size in *H. illucens* largely preserves proportional morphology while maintaining subtle allometric effects on wing shape. We discuss these findings as a quantitative basis for incorporating allometric constraints into industrial breeding strategies aimed at optimising black soldier fly performance for sustainable mass-rearing systems.

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Evidence of frequency-dependent stress responses in *Tenebrio molitor* larvae: implications for welfare in rearing systems

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Recent studies indicate that *Tenebrio molitor* larvae exhibit behavioural flexibility and context-dependent responses that go beyond simple reflexive patterns, supporting the hypothesis that this species may possess basic intentional-like capacities even at the larval stage. These findings raise important questions regarding how standard husbandry practices may affect larval welfare, particularly in large-scale and semi-intensive rearing systems. Mechanical vibrations are routinely generated during common management procedures, including substrate replacement, container handling, mechanical feeding systems, and environmental maintenance. Despite their widespread use, the potential role of vibrational stimuli as stressors for insect larvae has received little empirical attention. In this study, we investigated the behavioural responses of *T. molitor* larvae to controlled vibrational stimuli, with a focus on frequencies relevant to those produced by typical rearing equipment. Larvae were exposed to mechanical vibrations across a range of frequencies, and freezing behaviour, a stress-related behavioural indicator, was quantified. Results suggest that vibrations already at around 25 Hz induce a significant increase in freezing responses, consistent with a heightened state of alertness or stress sensitization. This pattern indicates that low-frequency vibrations may carry a negative valence for larvae and are not behaviourally neutral. Importantly, these frequencies overlap with those generated by everyday rearing activities. Although the present data are exploratory, they highlight the need to consider vibrational exposure as a potentially relevant factor in insect welfare. Further investigation is required to clarify frequency-specific thresholds, long-term effects, and possible mitigation strategies. Incorporating sensory ecology and behavioural responsiveness into rearing protocols may contribute to more welfare-oriented and sustainable insect production systems.

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Nanoparticle-mediated CRISPR/Cas9 delivery in *Hermetia illucens* for enzymatic PET degradation

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Polyethylene terephthalate (PET) is among the most extensively used synthetic polymers and represents a major source of persistent plastic pollution due to its high chemical stability and resistance to natural degradation. The accumulation of PET-derived micro- and nanoplastics in organic waste streams highlights the need for innovative and sustainable degradation strategies. PET-hydrolysing enzymes, such as PETases, provide a promising biological approach for polymer depolymerization under mild conditions. In this study, we explore a nanotechnology-based gene delivery strategy to enable PETase expression in the larvae of *Hermetia illucens*, an insect species widely used for organic waste bioconversion. Biodegradable chitosan-based nanocarriers were developed for the encapsulation and delivery of CRISPR/Cas9 components. Nanocapsules were produced using ionic gelation or water-in-oil emulsification methods, and formulation parameters were optimised to enhance nucleic acid encapsulation efficiency. The resulting nanocarriers were characterised in terms of hydrodynamic size, morphology, colloidal stability, and controlled release behaviour. Ongoing and future investigations will assess transfection efficiency in insect cell cultures through fluorescence-based uptake studies, molecular analyses to detect CRISPR-induced genome modifications, and enzymatic assays to evaluate PETase activity. This work aims to establish a versatile and biocompatible platform for gene delivery in insects and to assess the feasibility of engineering *H. illucens* as a biological system capable of contributing to PET degradation. By integrating nanomaterials science, molecular biotechnology, and insect physiology, this approach lays the groundwork for innovative solutions in sustainable plastic waste bioprocessing. This project has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101204748

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Biochar for animal feed – can we use it for insects?

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Biochar, a carbon-rich material produced by pyrolysing biomass, is increasingly explored as a feed additive in animal husbandry. Across ruminants, pigs, poultry and fish, inclusion rates of roughly 0.1–4% of daily feed intake have been associated with improved growth performance, feed conversion, product quality (meat, milk, eggs), and enhanced resistance to gut pathogens (Nair *et al.*, 2023). Adding biochar to the feed of conventional farm animals has been practiced for a very long time. Cato the Elder mentioned this use in the context of treating oxen, a 1906 Animal Husbandry Manual also noted that pigs naturally tend to consume small pieces of coal. Similar behavior has been observed in some wild animals, which voluntarily ingest charcoal fragments (Schmidt *et al.*, 2019). Studies on animals raised with biochar supplementation (including cattle, goats, pigs, chickens, ducks, and fish) report increased body weight and reduced feed conversion ratio (FCR), indicating greater biomass gains and more efficient feed utilisation (Man *et al.*, 2020). In relation to insects, research focuses less on biochar as a nutrient and more on its biological effects. When mixed directly into artificial diets or pupation media, biochar generally suppresses growth, survival, and successful metamorphosis in lepidopteran pests such as *Manduca sexta* and *Spodoptera frugiperda* (Wagle *et al.*, 2025). However, is there a possibility to use it in insect reared for feed and food, to improve its development? This work will try to summarize what we know so far about the use of biochar for insect feeding. This work was funded by the National Science Centre, Poland, Opus 28 number 2024/55/B/NZ9/02474. References: Man, K.Y., Chow, K.L., Man, Y.B., Mo, W.Y. and Wong, M.H., 2021. Use of biochar as feed supplements for animal farming. Critical Reviews in Environmental Science and Technology 51: 187–217. https://doi.org/10.1080/10643389.2020.1721980. Nair, P.S., P S, S.M., Suresh, S., A J, S., K, S., S, A.K., P R, A., Krishnan, N., S, P., Chakraborty, S., Chopra, H., Akash, S., Amin, R., Dey, A., Alagawany, M., Chandran, D. and Dhama, K. (2023). Beneficial impacts of biochar as a potential feed additive in animal husbandry. Journal of Experimental Biology and Agricultural Sciences 11: 479–499. https://doi.org/10.18006/2023.11(3).479.499. Schmidt H.-P., Hagemann, N., Draper, K. and Kammann, C., 2019. The use of biochar in animal feeding. PeerJ 7: e7373. Wagle N., Unnikrishnan, S., Kaur, S., Pereira, E. and Kariyat, R., 2025. Biochar suppresses growth, pupation and eclosion success of a specialist (*Manduca sexta*) and a generalist (*Spodoptera frugiperda*) insect herbivore. Journal of Sustainable Agriculture and Environment 4: e70069.

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The effect of black soldier fly larvae inclusion on hepatic gene expression in Bianca di Saluzzo chicken breed

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The chicken industry is significantly dependent on soybean meal as it is a crucial protein source. Alternative feeding ingredients, like soy-free vegetal protein sources and black soldier fly larvae (BSFL), are in high demand due to their favourable nutritional profile (1). This study evaluated the effects of BSFL and soy-free vegetal meal replacements on hepatic gene expression in the slow growing Bianca di Saluzzo chicken breed. A total of 192 male chickens were randomly allocated to 24 pens (8 birds each) and four dietary groups (6 replicates/diet): Control (C), Soy-Free (SF): replace soybean with vegetal protein, dried larvae (DL): SF with 5% dried BSFL supplemented, and live larvae (LL): SF with 5% live BSFL supplemented (2). 96 liver samples were collected at 147 and 174 days (each time 48 birds, 2 birds per each replicate) for RNA extraction. Targeted RNA sequencing focused on genes related to stress response (*CASP6*, *CAT*, *HSPA2*, *SOD1*) and lipid metabolism (*ACOX1*, *FABP1*, *LPL*, *SREBF2*, *FADS2*). Substituting soybean meal did not negatively affect the lipid metabolism gene expression. DL and LL increased *HSPA2* and *CASP6* with no differences between them. Expression of these two genes was upregulated at older age in all dietary groups, indicating physiological changes related to sexual maturity and meat composition rather than stress caused by feed (3). In older insect-fed birds, *CAT*, *SOD1* and *SREBF2* were upregulated, while *SOD1* was uniquely upregulated in LL, suggesting increased antioxidant resilience and lipid metabolism associated with weight gain in elderly birds (4). In conclusion, replacing soybean meal with alternative protein sources yielded comparable molecular outcomes to conventional diets. Insect-larvae supplemented diets mildly activated cellular stress and apoptotic pathways in young chickens, likely reflecting early physiological development. Both DL and LL demonstrated equivalent molecular effects, confirming their similar functional potential as sustainable protein ingredients in poultry nutrition.

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Selection of bacterial probiotics to enhance bioconversion ability and growth performance of mass-reared insects

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In recent years insect farming has gained great attention to bioconvert agro-industrial by-products into valuable biomass in the context of circular economy and to partially or fully substitute conventional protein sources used in feed and food systems. Insects have established intricate associations with diverse microbial partners which play crucial roles in host physiological processes, defence, and overall fitness. Gut eubiosis is critical for host health and physiological homeostasis, as alterations of the gut microbial structure may negatively affect host health and physiological functions. Thus, supplementation of beneficial bacteria may help insects improve their growth, bioconversion ability and health in general. Aim of this work was to characterise the cultivable microbiota associated with the yellow mealworm (YM) *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) to select candidate probiotics that can promote host growth and health, particularly when insects are reared on different agro-industrial by-products. To obtain a detailed description of the gut bacterial community associated to the insects, 16S rRNA gene amplicon sequencing was performed. Guts were dissected from surface-sterilized insects and used for bacterial isolation on selective culture media. The resulting bacterial collections were dereplicated by ITS-PCR fingerprinting and taxonomically identified through 16S rRNA gene sequencing. The isolates were further evaluated for their ability to hydrolyse key dietary polysaccharides, including cellulose, starch, and pectin, which can be found in agro-industrial by-products, as well as for their antimicrobial activity against entomopathogenic microorganisms. Overall, this study provides a comprehensive characterisation of the gut microbiota of an edible insect species, with particular emphasis on its cultivable fraction. This approach enables the identification of potentially beneficial bacterial strains that may be exploited in microbially assisted rearing strategies to enhance insect production and promote the bioconversion of organic wastes into high-quality products. Work funded by PRIN 2022 Project InProFarm (Insect Probiotic-assisted Farming: a promising tool to enhance edible insect health and performances), grant number 2022L4NJMK, European Union — Next Generation EU, Mission 4, Component 1.

INSECT GENETICS, PHYSIOLOGY, HEALTH AND WELFARE

Macronutrient profile shapes mealworm life history, but not its microbiome

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The mealworm (*Tenebrio molitor*) is an important industrial insect that is commonly reared as a high-quality protein source for pet food and livestock. Additionally, there is a growing interest in their capability to digest plastics like polystyrene. Therefore, efforts to understand the effect that diet has on the mealworm and its gut microbiome are important to determine its performance under varying nutritional contexts and bolster production systems. In this study, we measured survival and weight of mealworms that were reared on three different synthetic diets: a carbohydrate-rich diet (1P:3C), a balanced diet (1P:1C), and a protein-rich diet (3P:1C). We found that mealworm survival and weight were highest when reared on the protein-rich diet. To understand the impact of these diets on the mealworm microbiome, we conducted metatranscriptomic sequencing on mealworm gut tissues. We found that these diets did not have large impacts on the microbial community, since differences in alpha and beta diversity were not significant. However, we found 40 differentially expressed microbial transcripts that were upregulated in the balanced diet but downregulated in the carbohydrate and protein-rich diets. Interestingly, we detected *Zophobas morio* black wasting virus in five of our 12 samples, which was responsible for a recent epidemic in superworms (*Zophobas morio*) and could have implications for safe rearing practices in facilities with both mealworms and superworms. Overall, our results suggest that protein-rich diets can increase mealworm performance, but these improvements are not necessarily related to the gut microbiota.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Linking biological performance to economic outcomes: a sensitivity analysis of *Acheta domesticus* rearing

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Despite the industry's promise, economic viability remains a persistent hurdle for ventures of all scales. This challenge is exacerbated by a lack of unified quantitative frameworks linking biological metrics (growth, survival) with financial outcomes. This disconnect limits researchers' ability to determine if biological gains observed in empirical trials translate into actual economic returns, and prevents farmers from identifying which biological traits to prioritize for profitability. We developed an open-source bioeconomic model to bridge these gaps for *Acheta domesticus*. Calibrated using empirical datasets, the model integrates functions for survivorship, logistic growth, and age-dependent feed conversion. This framework simulates batch production dynamics to determine the harvest timing that maximises annualized profit per square area. Our sensitivity analyses reveal that profitability drivers are not universal. Instead, economic outcomes are sensitive to specific parameters contingent on a farmed population's specific life history traits. Furthermore, we demonstrate that the optimal harvest window is dynamic, shifting significantly in response to biological performance and local cost structures. This work proposes a standardized baseline for bioeconomic benchmarking. By quantifying the relative economic weight of different biological traits, the framework allows researchers to prioritize high-leverage targets for selective breeding and management. Crucially, it provides a flexible tool for producers to generate personalized insights aligned with their unique biological performance and cost constraints. The model is designed to accommodate future extensions of relevant covariates such as density, temperature, and feed compositions, or stochastic risk factors such as pathogen-driven colony collapse to reflect the complexity and volatility of industry-scale production.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Techno-economic model of insect farming — viable scenarios and trade-offs

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As the global demand for sustainable protein alternatives intensifies, insect farming — specifically the production of black soldier fly larvae (BSFL) — has emerged as a frontrunner in the circular bioeconomy. However, the industry faces significant hurdles regarding high capital expenditure (CAPEX) and volatile operational costs. A comprehensive Framework for Techno-Economic Modelling (TEM) designed to evaluate the financial viability and industrial scalability of insect protein production systems is proposed. The framework integrates mass-balance equations, CAPEX and OPEX assumptions with discounted cash flow (DCF) analysis to quantify the relationship between biological performance (e.g., feed conversion ratios, larval growth rates) and economic indicators such as Net Present Value (NPV). Central to this model is a multi-objective optimisation layer that identifies critical trade-offs between automation levels and labour costs. While high-tech environments may optimise yields, the initial investment often extends the payback period beyond viable commercial thresholds. Furthermore, the study conducts a Viable Scenario Analysis across distinct market price assumptions and facility capacities. By utilising sensitivity analysis, we assess how fluctuations in cost factors such as energy prices and substrate (organic waste) consistency impact long-term profitability. Results indicate that the "decentralized-hybrid" model—combining low-tech waste processing with centralized refining—yields the most robust economic resilience. This framework provides stakeholders, from agri-investors, operators to policy makers, with a standardized tool to evaluate investments and optimise the resource-efficiency of insect-based value chains.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effects of adult density on egg production and neonatal output in industrial-scale black soldier fly rearing

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Optimising adult rearing conditions is critical for maximising egg production and neonatal output in industrial-scale black soldier fly (BSF, *Hermetia illucens* L.) systems. While larval rearing has been extensively studied, adult reproductive performance remains a key bottleneck strongly influenced by adult density. This study evaluated the effects of adult density on reproductive output in a proprietary industrial batch-rearing system. Six adult densities (15 000, 25 000, 30 000, 35 000, 45 000 and 48 000 adults per cage) were tested in 1.42 m³ cage under controlled conditions (27 °C, 70% RH, 12:12 h LED photoperiod). Automated hydration (three times per day) and a proprietary oviposition attractant were provided; Pupae were introduced 1–2 days prior to adult emergence, and each density was replicated three times over a six-month period. Eggs were collected daily over a 10–12-day production cycle corresponding to peak oviposition. Performance indicators included total egg production per cage and batch, eggs per female (assuming a 1:1 sex ratio and supported by preliminary observations), adult emergence rate, and cumulative neonatal yield per batch; total egg production and eggs per female were analysed using one-way ANOVA. Total egg production peaked at intermediate densities, with cages stocked at 25 000–30 000 adults yielding significantly higher egg output per cage (199 ± 4 g at 30 000 and 184.33 ± 2.30 g at 25 000) than both lower densities (100.67 ± 9.71 g at 15 000) and higher densities (170.67 ± 9.45 g at 35 000, 157.33 ± 5.50 g at 45 000 and 125.67 ± 3.79 g at 48 000). Eggs per female were highest at 25 000 adults (3.97 ± 0.04 mg) indicating reduced reproductive efficiency at both lower and higher densities. Adult emergence exceeded 90% across all treatments, and cumulative neonatal yield remained consistent at approx. 31% across all densities, indicating stable hatching performance regardless of adult density. Overall, intermediate adult densities (25 000–30 000) maximised both total egg output and per-female efficiency, while lower densities limited mating opportunities and higher densities reduced efficiency due to high-density constraints. Consistent neonatal yields suggest density-dependent effects primarily act on adult reproductive performance rather than egg viability. Defining optimal adult density thresholds is thus essential for improving efficiency and scalability in industrial BSF production.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Black soldier fly *H. illucens* farming in rural Greece under variable temperature regimes and local agrifood residues

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Insects will only feed the world if their farming can be implemented cost-effectively and sustainably across the globe. Thus, rural small/ medium-scale applications are at least as important as industrial ones. In Europe, the mild climate and unvalorised agrifood residues of the Mediterranean region can allow insects to be integrated into existing farm structures with minimal active climate control and feedstock-costs. To demonstrate the potential of such context, we took as a case, black soldier fly *H. illucens* (BSF) farming in rural Greece. We focused on two aspects that are key to cost-effectiveness and sustainability: climatisation and feedstock. *Energy-efficient BSF farming under variable temperature regimes in a Mediterranean greenhouse*: We conducted BSF larval growth experiments in industry-type rearing trays using brewer's spent grain (BSG) as feedstock. The experiments took place July 2024-December 2025 in an experimental greenhouse in southern Greece, with minimal climatisation, mimicking commercial horticulture greenhouses of the region. Larval growth and bioconversion performance as well as the effects of rearing parameters on these were in line with findings under more tightly controlled conditions: Individual larval growth improved but the efficiency of bioconversion decreased with increasing feed availability per larva, while the feed dry matter content was a key source of variation. The unstable ambient temperature enabled us to pinpoint the boundary conditions for bioconversion under variable temperature regimes, which, to our knowledge has not been previously studied. Based on this, we estimated the energy demand for maintaining permissive variable temperature regimes for year-round BSF farming in a Mediterranean greenhouse, in comparison to the respective energy demands in other settings with constant temperatures. Unvalorised Mediterranean agrifood residues as BSF larva feedstock: In the same greenhouse setting, we incorporated local agrifood residues as feedstock, focusing on materials which, due to physicochemical properties or logistics were otherwise unvalorised. Particular combinations of olive mill residue, cheese whey, raw dough, bakery rejects, greenhouse crop residues and BSG were efficiently bioconverted. We present an outlook on how these partially seasonal materials can be stored and pre-processed with minimal energy use to be integrated into year-round operation at a meaningful scale. Overall, these results concerning climatisation and feedstock highlight rural Greece in particular, and the Mediterranean region in general as a promising context for cost-effective, sustainable BSF farming.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Beyond biomass: microbial stabilization of host homeostasis in *Hermetia illucens*

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Understanding the tripartite interactions among host, diet, and microbiome is fundamental to optimising black soldier fly (*Hermetia illucens*, BSF) production at scale. While research often prioritizes microbial drivers of absolute biomass, this study utilises the BSF as a model of physiological resilience. We demonstrate how specific microbes act as "biotic filters" to stabilize host development, ensuring predictable outcomes even when a biological ceiling prevents further increases in mass. By enriching gut microbes from high-performing larvae, we identified *K. aerogenes* as a microbial "landmark" associated with peak performance. We then probed this landmark by supplementing high-dose *K. aerogenes* into an already balanced rearing substrate. Because the control larvae were performing at peak physiological efficiency, the supplement did not significantly increase absolute mass. However, the addition of *K. aerogenes* triggered a profound stabilization of the phenotype, resulting in an 80% decrease in weight variation (Coefficient of Variation) compared to the control group. Mechanistically, *K. aerogenes* establishes niche dominance within the gut, acting as a biotic filter that causes a functional crash of the resident fungal community. This bacterial-fungal competition governs the stability of the internal environment, which we further validated through host transcriptomics. We identified 52 differentially expressed host transcripts pointing to a process of "molecular housekeeping," suggesting that the host "sensed" the stabilized microbial environment and synchronized its metabolism toward internal homeostasis. The significance of this work lies in its shift from the "bigger is better" narrative toward a focus on physiological stability. In industrial bioconversion, achieving population uniformity is a primary hurdle for predictable scaling and economic efficiency. This study provides a mechanistic blueprint for standardizing larval output through microbial niche gatekeeping, offering a new ecological lens for probiotic interventions in sustainable protein production.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Dietary sugars influence substrate temperature and larval growth in black soldier fly larvae bioconversion

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Substrate temperature is a key factor for climate control and larval growth in black soldier fly larvae (BSFL) bioconversion. Excessive heat interrupts operation, yet its drivers are understudied. Digestible carbohydrates, often a highly variable and abundant component of organic waste substrates in BSFL, could be a critical driver of temperature variation. Therefore, this study investigated whether different digestible carbohydrates in organic waste affect temperature and larval growth. In a 7-day controlled feeding trial with industrial crates (60 × 40 cm crates, 8,640 larvae each), we added carbohydrates to the substrate (20% based on dry matter) to determine whether the sugar type (isomaltulose versus sucrose, i.e. high versus low digestion rate) and availability (day 0, day 3) affected larval dry mass, substrate temperature, metabolites, and pH, CO_2, and NH_3 emissions. We found that sugars strongly altered substrate temperature in a time-dependent manner. Sucrose addition on day 0 produced higher temperatures (mean increase: 2.1 °C, max.: 7.9 °C) and resulted in a lower final larval mass (approx. 20%) compared to sucrose addition on day 3. Isomaltulose addition resulted in a lower mean (0.4 °C versus sucrose) but highest peak (51.3 °C) temperature. pH and NH_3 concentration dropped after sucrose addition. Trends in metabolites suggested O_2-dependent metabolic shifts during the early bioconversion. These results showed that initial amounts of digestible sugars in organic wastes can lead to elevated temperature peaks early in the bioconversion process and lower larval yield. The study improves the understanding of heat generation in food waste recycling with BSFL and suggests that tailored, sequential feeding strategies could optimise substrate temperatures, larval yield and environmental process performance.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effects of early larval weight on survival of *Hermetia illucens* across waste substrates

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BSF farming has become increasingly optimised, yet key uncertainties remain regarding management of the nursery stage. In particular, there is no consensus on when larvae should be transferred from nursery substrates to the waste feedstock. Current recommendations are typically based on larval age, despite substantial variation in early growth rates under different rearing conditions. To improve the comparability of studies and farming practices, evaluating the effect of early larval weight on survival is crucial. This study was conducted at SLU to evaluate whether early larval weight provides a robust criteria for the successful transfer of BSF larvae to waste substrates. Prior to the start of the experiment, approx. 100 000 neonate larvae originating from a single egg cohort, all hatched within an 8-hour window, were reared on chicken feed (CF) provided ad libitum until they reached a size that permitted manual handling and counting. Throughout the experiment, the cohort was maintained parallel under the same controlled laboratory conditions (29 ± 1.5 °C, RH 40%) as the following waste treatment. The experiment began when the individual average weight of the seed larvae was 0.13 mg. On the first day, the first waste treatments were established, using a set amount of hand counted larvae per replicate. New groups of progressively heavier larvae were collected from the same cohort and introduced into the same waste source in triplicates every 24 h. This approach resulted in waste treatments containing larvae of increasing initial weight over 5 consecutive days, allowing assessment of early-stage mortality across a range of seed larval sizes. Each waste stream triplicate started on different larva sizes was terminated after seven days, and the survival of the larvae was checked. Using this approach, we evaluated larval survival across commonly used substrates (CF and food waste (FW)) as well as more challenging wastes, including fermented FW with/without insect frass, orange and cabbage cuttings, and mussel meat (MM). The results of this experiment identified a minimum weight threshold for successful establishment and results show that across most waste types, larvae weighing approximately 0.4 mg could be transferred without negatively affecting survival. Reduced survival was observed only on particularly tough substrates, such as high-protein MM but the use of heavier larvae (10 mg) increased the survival significantly. These findings demonstrate that larval weight, rather than age, is a key determinant of early survival and provide a practical, weight-based criteria for optimising nursery duration and larval transfer in BSF production systems

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Achieving thermogenesis in bench-scale black soldier fly larvae experiments

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Reproducibility of black soldier fly larvae (BSFL) rearing results within and among locations, and especially when scaling from bench scale to industrial scale, remains a challenge. One factor that has so far been insufficiently considered is the accurate mimicking of substrate temperature. Substrate temperature can rise above ambient due to accumulated metabolic heat from larvae and microbes, a phenomenon termed thermogenesis. Among others, this effect depends on substrate and larval mass and the corresponding surface area-to-volume ratio. However, BSFL research rarely accounts for scale-dependent thermogenesis in experimental designs, complicating interpretation and replication of results. Specifically, thermogenesis in small-scale laboratory experiments (low substrate/larval mass, higher surface area-to-volume ratio) rarely mimics thermogenesis in large-scale industrial settings (high substrate/larval mass, lower surface area-to-volume ratio). We hypothesized that lower temperatures in small-scale experiments are caused by elevated heat loss, and that this can be overcome using insulated rearing containers. We tested this hypothesis in controlled bench-scale rearing experiments. Insulated and uninsulated stainless steel vessels (12 cm diameter, 1100 larvae each, 700 g chicken feed-based diet at 28% dry matter) were compared in a 7 day experiment including six replicates per treatment. During the experiment substrate temperature was measured every 5 minutes using Testo 175 T3 loggers (Testo, Germany) with external thermocouple probes. After harvest the individual larval weight, total larvae weight, frass total weight, and dry matter content for both biomass fractions were measured. Data was analysed with Anova and post hoc testing. Results demonstrated that insulated rearing containers effectively minimised biological heat loss in small-scale experiments. Insulated vessels showed greater temperature increase above ambient compared to uninsulated vessels (mean increase 15.7 °C vs. 5.4 °C), with higher peak temperature (50.5 °C vs. 37 °C). This suggests that insulation alters the energy balance, and heat accumulates when loss rate to the environment is lower than production rate. High temperatures in the insulated containers seemed to mirror the phenomenon of overheating, which is common with large substrate mass (9–40 kg) in BSFL rearing, where the substrate insulates itself. This approach could improve consistency and replicability of BSFL rearing experiments, while increasing the predictability of bench-scale to industrial-scale results.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Biowaste treatment using black soldier fly larvae: effect of substrate macronutrients on process performance

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Black soldier fly larvae (BSFL) are widely used for the treatment of solid organic waste by converting biowaste into a protein- and lipid-rich larval biomass. Process performance, evaluated with survival rate (SR, %) and waste reduction efficiency (RE, % dry matter), depends on substrate nutritional quality. Substrate quality can be described by the overall macronutrient concentration ($\sum$PCL), defined as the sum of proteins (P), lipids (L) and non-fibre carbohydrates (NFC), and by the relative abundance of a single macronutrient (XP, XNFC, XL). This study quantitatively combines published literature to assess how substrate quality affects BSFL process performance. A systematic literature review was conducted following the PRISMA methodology. Data on substrate macronutrient composition and process performance were extracted from 35 peer-reviewed studies (83% Q1), covering 177 substrates, including biowaste and artificial diets. Substrates were classified into six categories: meat and fish waste, manure, grain waste, food waste, vegetables and fruits, and artificial substrates. Most substrates showed high nutritional quality, with 133 substrates exhibiting $\sum$PCL > 50% and 124 substrates exhibiting balanced macronutrient ratios (XNFC > XP > XL). These substrates were mainly food waste, vegetables and fruits, and artificial diets. In contrast, data on low-quality and unbalanced substrates, such as meat and fish waste, were limited. SR is independent of $\sum$PCL, as most substrates achieved SR ≥ 80%. However, SR is influenced by macronutrient ratios and decreases under lipid excess (XL > 0.6), protein deficiency (XP ≤ 0.05), or protein excess (XP > 0.5) combined with low NFC (XNFC < 0.15). RE, in contrast, depends on $\sum$PCL. On low-concentrated substrates ($\sum$PCL < 60%), RE decreases below 40%, especially in the case of lipid excess (XL > 0.65) and/or protein imbalance (XP ≤ 0.05 or XP > 0.5 with XNFC < 0.20). Highly concentrated substrates with $\sum$PCL > 60% generally achieve RE ≥ 40%, which further increases when macronutrients are balanced, with XP > 0.6 and XNFC > XL. By integrating both SR and RE data, good-quality substrates for BSFL treatment were identified (when SR ≥ 80% and RE ≥ 40%) based on the relative macronutrient abundances. The results can be used as a simple tool for rapid evaluation of individual substrate suitability for the BSFL process and for identifying the optimal substrate mixture to improve process performance.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Batch-invariant identification of edible insects from NIR spectra with explainable AI

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Reliable authentication of edible-insect species is essential for the safety, traceability, and regulatory compliance of emerging insect-based food and feed supply chains to prevent food fraud and process inefficiencies. Near-infrared (NIR) spectroscopy offers a fast, non-destructive way for automated species verification, but its reliability is often undermined by data shift that occurs when spectra are collected under different real-world conditions. Strong batch effects due to variation between production runs and insect processing-induced spectral distortions can alter signals, scattering behaviour, and the intensities of biochemical absorption bands. To address this, we introduce the Batch-Invariant Spectral Transformer (BIST), a batch-generalizing model that jointly learns a spectral preprocessing module and a species classifier while using an adversarial branch to suppress batch-discriminative information. We use a full-factorial dataset of 2,700 spectra, spanning three insect species (*Acheta domesticus*, *Hermetia illucens*, *Tenebrio molitor*), three batches, three processing treatments (tap water, blanched with tap water, plasma-activated water), and two ultrasound conditions (with and without ultrasound). This dataset enables comprehensive evaluation of BIST under leave-one-batch-out (LOBO) protocol. BIST achieves 0.93 ± 0.04 accuracy, outperforming classical chemometric models by 9–39% and recent deep spectral models by 5–41%, while producing batch-invariant, biochemically meaningful representations. We integrate a gradient-based counterfactual framework to BIST that quantifies the minimal spectral adjustments required for a sample to be reassigned to its true insect class. Spectral region-restricted counterfactuals, defined across twelve chemically curated NIR bands, show that lipid- and protein-dominated regions provide the causal leverage necessary for true species recovery. These counterfactual signatures align with established biochemical differences among the insects and clarify the mechanisms underlying systematic misclassification under specific processing conditions. Together, these analyses demonstrate that BIST achieves robust cross-batch generalization while presenting interpretable biochemical structure and spectral region-level insight, establishing a reliable foundation for NIR-based species authentication in industrial settings.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Optimising larval density in black soldier fly (BSF) production using a dry hatching system

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Efficient and scalable techniques for black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larval production are essential for advancing sustainable waste management and providing alternative protein sources. This study presents the implementation and qualification of a dry hatching system for BSF neonate collection and incubation at nextProtein's industrial production facility. The case study shares nextProtein's experience transitioning to a new production process while maintaining consistent performance at scale. Since its founding in 2015, nextProtein has relied on a simple reproduction process: collecting fresh eggs daily and directly dosing them into crates supplied with fresh feed. While effective and low-cost in early operations, this method introduced major challenges in later stages due to high heterogeneity in larval weight and density both within and between crates. Based on preliminary tests, results and consulting recommendations, a dry hatching process was designed and all related equipment and devices were developed or sourced to ensure its industrialisation following a strict phasing plan. First, the weighing of the egg collectors ("eggies") is done before they are placed into the cages. Then, the following day, these eggies are weighed again to determine the mass of the eggs. After 24 h, these same egg collectors are transferred to specially designed equipment aiming to regularly collect the newly hatched larvae for 5 days within a precisely controlled environment (temperature & humidity). The system demonstrated high efficiency in guiding newly hatched larvae toward targeted collection areas, reducing losses from hatching through early larval stages and reducing the total growth time before harvest. The process allowed an important hatching rate of 92%, an average of neonates weight of 0.0159 mg an average weight after incubation (5 DOLS) of 4.9 mg with an overall survival rate of 94%. Following implementation, larval yield doubled for an equivalent egg input, while reducing the fattening phase duration by 20%. The findings highlight the potential for scalable applications of dry hatching systems in BSF production, particularly for operations aiming to improve biosecurity, resource efficiency and productivity. Further investigations will focus on refining incubation conditions and scaling the system for larger industrial setups.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effect of high-intensity ultrasound on quality of proteins from three different insect species

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The low acceptance of insects among consumers suggests that enriching food products with extracted insect proteins could be a useful approach. Ultrasound processing (US) can improve the protein extraction yield. However, little is known on the impact of US on the quality of the proteins. In the current study, three insect species (*Tenebrio molitor* (Tm), *Hermetia illucens* (Hi), *Acheta domesticus* (Ad)) and three independent batches were investigated. Frozen insects were mixed with water and reduced into paste by applying three different treatments (A, tap water; B, blanching (70 °C, 5 min) + tap water; C, plasma water). Obtained pastes were treated with US (700 W, 30 min, 5 s ON–55 s OFF) and the proteins were extracted. Techno-functional properties (water holding capacity (WHC); oil holding capacity (OHC)), oxidation status (number of thiol, disulphide and carbonyl groups) and biological activities (antimicrobial and antioxidant capacity) were investigated. Results showed that Ad had the highest WHC (3.35 ± 0.19%), regardless of the treatment and the US process. No significant differences were observed in OHC. Number of thiols, disulphides and carbonyls were significantly affected by species and treatment. The highest thiols were always detected in Hi (39.19 ± 1.70 μmol SH/g protein), followed by Tm (20.26 ± 1.61 μmol SH/g protein) and Ad (12.10 ± 0.92 μmol SH/g protein). US led to an increase of thiols in Tm-A (+2.83 μmol SH/g protein), while they decreased in Tm-B (–2.61 μmol SH/g protein) and Hi-C (–4.22 μmol SH/g protein). US reduced the number of disulphides in Tm (–84.96 μmol SS/g protein), while increased them in Ad (+40.44 μmol SH/g protein) and Hi (+44.45 μmol SH/g protein). Number of carbonyls increased in Tm-C (+0.36 μmol COO/g protein) and Ad-C (+0.82 μmol COO/g protein), while decreased in Tm-B (–0.42 μmol COO/g protein) and Ad-B (–0.16 μmol COO/g protein) after US. Application of US always led to an increase of antioxidant activity (FRAP: +1.09 μmol TE/mg protein, ABTS: +8.60 μmol TE/mg protein). Antimicrobial activity against *Escherichia coli* was only noticed in Hi-A and disappeared after US. Activity against *Salmonella enterica* was recorded in Ad and Hi and boosted by US. Antimicrobial activity toward *Staphylococcus aureus* was observed in Tm. In summary, this study shows that protein extracts from different insect species have significantly different biological and techno-functional properties and that these activities can be influenced by the processing method used.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

From insect proteins to food systems: a stepwise processing and mimicking strategy for edible insect-based foods

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Edible insects are increasingly recognized as sustainable alternative protein sources; however, their application in food systems remains limited by poor solubility, restricted techno-functionality, and low compatibility with conventional food matrices. This study investigates a stepwise processing and mimicking strategy to systematically transform mealworm (*Tenebrio molitor* larvae) proteins into food-ready ingredients by sequentially aligning their functional and structural properties with those of conventional food proteins. First, the effects of extraction strategies on health-related functionality were evaluated using conventional (alkali and salt) and non-conventional (enzyme and screw-press) methods. Enzyme- and salt-extracted protein concentrates significantly enhanced insulin signalling in cell-based models, increasing p-Akt/Akt ratios by approximately 2.3–2.7-fold and 1.3–1.6-fold, respectively, compared with untreated controls. In addition, bioactive peptides derived from mealworm protein hydrolysates (FDKY and FDRL) significantly suppressed myostatin expression and downregulated muscle atrophy markers under dexamethasone-induced conditions, indicating tunable anti-diabetic and anti-muscle atrophy potential through extraction and hydrolysis design. To improve techno-functional performance, physicochemical processing combining heat treatment, pH-shifting, and high-pressure homogenization (HP + HT + PS) was applied to mealworm flour. This integrated treatment increased protein solubility from 0.23 to 7.50 mg/ml and enhanced surface hydrophobicity by up to 85-fold, resulting in improved emulsifying activity, stability, and reduced droplet size. These improvements are critical for incorporating insect proteins into emulsion-based and liquid food systems. For further functional mimicry of conventional protein powders, mealworm protein isolate (MPI) was co-spray dried with gum Arabic (GA) or maltodextrin (MD). Co-spray-dried powders showed enhanced *in vitro* digestibility, with higher degrees of hydrolysis (MPI + GA: 55.8%; MPI + MD: 47.6%) compared with freeze-dried or spray-dried MPI alone. Finally, mealworm-derived proteins were incorporated into a tofu system as a representative soy-protein matrix. Partial substitution of soybean protein enabled the development of protein-enriched tofu with tailored structural and functional characteristics. Overall, these results demonstrate that edible insect proteins can be effectively converted into food-compatible ingredients through a processing-driven, stepwise mimicking strategy, supporting their practical application in sustainable and functional food systems.

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Diet effects on development, nutrition, safety, and gut microbiota of *Protaetia brevitarsis* larvae

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Protaetia brevitarsis larvae are traditionally consumed in Korea and are gaining global attention as a sustainable protein source. However, large-scale utilisation requires scientific validation of how feed substrates influence larval development, nutritional quality, safety, and gut microbiota. This study evaluated the effects of fermented oak sawdust (FOS) and fermented oyster mushroom cultivation by-products (OMCB) on larval performance and quality. Larvae were reared under controlled conditions and fed either FOS or OMCB throughout the larval stage. Developmental traits, including survival rate, body weight gain, and development period, were recorded. Proximate composition, amino acid profiles, fatty acids, minerals, and hazardous substances were analysed using standard AOAC and food code methods. Microbiological safety was assessed by detecting foodborne pathogens and heavy metals. Gut microbiota composition was analysed using 16S rRNA gene sequencing. Larvae fed OMCB showed significantly higher weight gain and shorter development periods compared to those fed FOS, while maintaining high survival rates. OMCB-fed larvae exhibited higher crude protein content and comparable essential amino acid profiles, with elevated levels of proline and unsaturated fatty acids. Heavy metals and pathogenic bacteria were below detection limits in both groups. Gut microbiota analysis revealed distinct community structures depending on feed source, with mushroom-based diets promoting higher bacterial diversity and enrichment of host-associated taxa involved in carbohydrate degradation. Feed substrate strongly influenced larval growth performance and gut microbial composition without compromising nutritional value or safety. Fermented mushroom by-products provided improved nutrient availability and supported beneficial gut microbiota, demonstrating advantages for sustainable insect production. Fermented oyster mushroom cultivation by-products are a safe and effective alternative feed for *Protaetia brevitars*is larvae, enhancing growth efficiency, nutritional quality and gut microbiota profiles. These findings support the use of agro-industrial by-products in edible insect production and contribute to sustainable circular bioeconomy strategies.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Impact of membrane separation on functional and bioactive properties of insect proteins

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Membrane based fractionation offers a low impact route to tailor protein functionality. We studied how mild alkalinization of protein solutions before ultrafiltration (UF) and cascade membrane systems modulate the techno functional/bioactive properties of *A. domesticus* (cricket) and *H. illucens* (black soldier fly; BSF) proteins. Cricket and BSF proteins were solubilized at pH 7 or 9.5 and processed in a single 30 kDa UF step. Cricket protein was also processed in two cascade systems: Config. A (0.2 μm MF, 500 kDa, 100 kDa, 30 kDa) and Config. B (500 kDa, 100 kDa, 30 kDa). Permeate flux, protein rejection, emulsifying activity, and foaming capacity were quantified for all permeates and retentates. Bioactivity of low molecular weight (<30 kDa) fractions from the single 30 kDa was assessed by collagenase inhibition and *in vitro* cell proliferation/cytotoxicity up to 48 h. Adjusting the solubilization pH from 7 to 9.5 altered the UF fractions obtained with the 30 kDa membrane for both cricket and BSF. While these pH shifts strongly affected techno functional properties, the most notable effects concerned bioactivity. Cricket permeate at pH 7 showed clear bioactive potential, combining collagenase inhibition with enhanced cell proliferation after 48 h, without any cytotoxicity — indicating suitability for cosmetic applications. By contrast, BSF protein solutions at both pH levels stimulated cell proliferation at 24 and 48 h without cytotoxic effects, revealing previously unreported bioactivity in low molecular weight (<30 kDa) BSF fractions. These findings highlight that both insect species can yield biologically active protein fractions only applying membrane separation without requiring hydrolysis. Cascade membrane processing showed that membrane sequence strongly affects efficiency and protein functionality. Configuration A (starting with microfiltration) maintained a higher permeate flux (approx. 40 $l \cdot h^{-1} \cdot m^{-2}$) and yielded a more balanced protein distribution with lower rejection, producing an MF retentate with strong emulsifying properties. Configuration B, starting with 500 kDa UF, resulted in a lower steady state flux (approx. 30 $l \cdot h^{-1} \cdot m^{-2}$) and earlier protein concentration, with >80% rejection at the 500 kDa step. Overall, both set-ups show that cascade membrane separation can tailor insect protein fractions, but membrane choice and sequence are critical for optimal performance and protein functionality

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Insect proteins as sustainable multifunctional emulsifiers for diverse colloidal food architectures

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Insect proteins offer a versatile platform for structuring a wide spectrum of food colloids with contrasting techno-functional demands. Microfluidic observations, used to map how formulation variables influence early droplet stability, showed that lesser mealworm protein concentrate (LMPC) performs comparably to whey protein, with monodisperse droplets (55–118 μm) and coalescence suppressed at 0.02% protein, while pH and oil fraction modulated dimer formation. These insights help define stability windows across different emulsion formats (Jayakumar *et al.*, 2023). Chemical modification further broadens functionality: covalent conjugation of LMPC with tannic or chlorogenic acid markedly increased antioxidant capacity (up to approx. 845 μmol TE/g) without impairing emulsifying activity (approx. 53–55%). In PUFA rich O/W emulsions, these conjugates substantially reduced lipid oxidation during accelerated storage (Ballon *et al.*, 2024). Such antioxidant emulsifiers also enable multicomponent systems. Double emulsions with plant derived polyphenols and linseed oil achieved high encapsulation efficiencies (≥67.5%) and small droplets (≤8.35 μm), while exhibiting enhanced oxidative stability when stabilized with LMPC tannic acid. These systems illustrate how insect protein emulsifiers can support bioactive protection and controlled release in functional foods (Camelo-Silva *et al.*, 2025). Insect proteins (native or heat-treated) also stabilize high-internal-phase emulsions, HIPEs, (80% oil; 2–3% protein) with solid like rheology and yield stresses of 255–399 Pa. These HIPEs show adequate viscosity, elasticity and self-supporting capacity for extrusion-based 3D printing, producing fat analogues that retain shape for at least nine days under refrigeration. Thermal treatment increases viscosity and structure stiffness, expanding the textural palette accessible to formulators (Ballon, *et al.*, 2025). Altogether, native, thermally treated and polyphenol-conjugated insect proteins provide a sustainable toolbox for designing antioxidant emulsifiers, bioactive-loaded double emulsions and printable fat-rich HIPEs, demonstrating their relevance for food applications requiring tailored interfacial and rheological functionalities.

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High-pressure–assisted protein extraction to modulate functionality in black soldier fly

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High-pressure-assisted extraction (HPAE) of edible insects remains underexplored. In this study, the potential of HPAE to produce black soldier fly (*Hermetia illucens*) protein extracts with tailored technological properties was investigated. High-pressure treatments at 100, 200, 300 and 400 MPa were applied for 5 min at 30 °C using a bench-scale high-pressure processor to organic solvent–defatted BSF fractions (0.8 g) under alkaline conditions (0.05, 0.10, 0.20, and 0.25 M NaOH) in the presence of 0.05 M NaCl. The alkaline condition, combined with low ion concentration (<0.05 M NaCl), was selected to enhance protein extraction. Protein extraction yield, protein molecular weight distribution, emulsifying activity, and emulsion zeta potential were analysed to assess the combined influence of pressure and alkaline extraction conditions on the functional properties of BSF protein extracts. HPAE significantly increased protein recovery ($p < 0.05$) compared with the control. With NaOH at 0.20 and 0.25 M, protein yield was significantly higher ($p < 0.05$), between 250 and 390 times that of control samples with 0.05 and 0.10 M NaOH being particularly enhanced at 200 and 300 MPa. Molecular weight distribution analysis indicated that HPAE increased the intensity of protein bands, though the distribution remained predominantly at 10–15 kDa, suggesting denaturation under these conditions. Nonetheless, samples suspended in 0.05 and 0.10 M NaOH and treated at 200 and 300 MPa showed a significant increase in protein bands at 100–150 kDa ($p < 0.05$), which were not found in the control samples. Emulsifying activity was higher in HPAE-treated samples compared with the control, and was particularly higher in samples suspended in 0.10 M NaOH and treated at 300 MPa. Zeta potential values of the emulsions were below –30 mV in all samples, including the control and HPAE-treated groups, showing the potential for producing stable emulsions from these protein extracts. This study demonstrates that HPAE can be effectively used to modulate the yield, molecular weight distribution, and functional properties of BSF protein extracts.

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Bringing order to complexity: performance monitoring at black soldier fly reproduction units

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Biowaste processing in low- and middle-income countries using black soldier fly (BSF) bioconversion has recently gained significant traction. To process the daily waste received, facilities ensure a steady supply of hatchlings in their reproduction units (RU). However, operators often struggle to maintain a stable and reliable RU. The complexity of the BSF life cycle combined with varying or sub-optimal operating procedures highly impacts their efficiency. As inefficiencies and disruptions in the RU impact the whole system, it is crucial to monitor key parameters and react with measures in a timely manner. Despite the importance of RUs, there is currently no consensus on monitoring methods nor on target performances among practitioners. We propose a BSF RU monitoring framework using four Reproductive Performance Indicators (RPI). The RPI-G contains information about the performance of the whole RU. The other three indicators: The RPI-P, RPI-E and RPI-N provide information about the larval, adult, and neonatal stages respectively. Using this framework, we analyse and compare nine existing RUs of bioconversion facilities around the world with different types of monitoring datasets. Subsequently, based on interviews with operators, we hypothesize possible associations between operating procedures and performance. The RPI framework was compatible with all 9 different datasets and data structures. Results show that there are large differences in performance among facilities, with variations in specific RPIs up to a factor of 4. Operational measures leading to increases in performance (by a factor of 2–3) were identified during interviews and are presented. We show that the RPI framework is near universal and enables comparison of a variety of RUs from different BSF facilities, independent of scale, operational protocol, and data collection methods. As RPIs are expressed in ratios, they can be calculated over multiple days, weeks or months, filtering out the day-to-day variability and offering an unbiased estimation of performance. This framework can be applied and implemented in RU monitoring, to identify a declining performance and simultaneously identify the source of the decline. The RPIs offer straightforward and interpretable performance measures for BSF RUs. They are robust to variability and can be used on historic data. This enables BSF practitioners to efficiently monitor their RU, to assess their RU quality, and to compare it with their peers'.

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Integration of emerging technologies for insect processing in a biorefinery framework

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This study assesses the integration of emerging processing technologies into an insect biorefinery framework, using yellow mealworm (*Tenebrio molitor*) larvae as a sustainable protein source. Effective upstream pretreatments are essential to ensure microbial safety, promote efficient fractionation, and preserve product quality. Instant Controlled Pressure Drop (DIC) and Pulsed Electric Fields (PEF) were therefore evaluated and compared with conventional hot-water blanching. Larvae were subjected to pretreatment followed by killing/decontamination, hydraulic pressing for simultaneous defatting and dewatering, and convective drying. Performance was assessed through microbiological analyses (total viable counts, yeasts and moulds), juice colour and browning, pressing yield, protein recovery, cake composition (dry matter and lipid content), protein structural changes (fluorescence spectroscopy), drying behaviour (shrinkage, effective water diffusivity, surface area, particle size distribution), and energy consumption. DIC pretreatment at 0.3 MPa for 15 s achieved rapid microbial reductions below European regulatory limits within seconds, outperforming blanching, which required several minutes, while PEF effectively killed larvae but showed limited microbial inactivation. DIC pretreatment also resulted in lighter juice colour due to enzymatic inactivation, whereas blanching and PEF promoted browning. Conventional blanching yielded 68% press juice, 48% protein recovery, and press cakes containing 45% dry matter and 14.7% lipids (dry basis). In contrast, DIC reduced juice yield to 55% but significantly enhanced protein retention in the press cake (78% recovery), with higher dry matter content (55%) and lower lipid levels (12% dry basis), likely due to protein aggregation. PEF increased juice yield (78%) but resulted in greater protein losses to the liquid fraction (43% recovery), with 54% dry matter and 14% lipids (dry basis) in the cake. DIC pretreatment substantially improved drying performance by reducing shrinkage, increasing effective water diffusivity, expanding surface area by 125%, and producing more uniform particle sizes. PEF also accelerated drying, though to a lesser extent than DIC. Energy consumption followed the order: PEF (21.7 kWh · t^{-1})<blanching (61.7 kWh · t^{-1})<DIC (111 kWh · t^{-1}). Overall, DIC and PEF offer complementary advantages within insect biorefinery systems, with DIC excelling in decontamination and drying enhancement, and PEF improving process efficiency and protein quality. Strategic integration of these technologies enables more sustainable and efficient insect processing pathways.

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An AI-based framework for feed optimisation in black soldier fly rearing under substrate variability

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Feed formulation is a key determinant of growth performance and biomass composition in black soldier fly (*Hermetia illucens*) rearing. Industrial optimisation is challenged by strong variability in organic waste substrates and the lack of structured methods to link feed composition, processing and biological response. This study presents an AI-based framework to systematically address feed optimisation under substrate heterogeneity. The feed formulation was modelled as a constrained, multi-objective mixture optimisation problem. The experimental feed classes included household vegetable waste, supermarket vegetable waste, agro-industrial residues (cassava pulp, palm oil decanter cake, and brewery spent grains), and fermented variants using Lactobacillus spp. A Material–Process–Biological response ontology was introduced to semantically structure substrate origin, pre-treatment, compositional descriptors, and larval response variables. Supervised machine learning models act as surrogate predictors, whereas Bayesian and evolutionary optimisation algorithms explore feasible feed combinations under cost, availability, and sustainability constraints. Simulation-based case studies using synthetic and literature-derived datasets showed that ontology-guided optimisation reduced the effective feed search space by approximately 40–60% compared to unstructured mixture exploration. Surrogate model benchmarking indicates predictive errors below 10–15% RMSE for growth-related target variables under realistic noise assumptions. Multi-objective optimisation yields Pareto fronts that explicitly quantify the trade-offs between growth rate, protein–lipid allocation, and feed cost, thereby enabling transparent decision support. The results demonstrate that combining formal knowledge representation with AI-driven optimisation improves robustness and interpretability when dealing with heterogeneous and seasonally variable substrates. The fermentation state and substrate class emerged as dominant explanatory factors in the optimisation landscape, highlighting the importance of structured data integration. The proposed framework provides a reproducible and extensible methodology for feed optimisation in BSF farming. By combining ontology-based knowledge modelling with AI optimisation, this study supports the scalable valorisation of heterogeneous organic residues and establishes a foundation for future experimental validation in industrial insect production systems.

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What's good enough? Bioconversion heuristics model based on mass balance and cost-benefit analysis

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Black Soldier Fly insect farming is a frontrunner among circular bio-economy solutions. To deliver on its positive impact potential, insect farms need to be profitable investments and deliver competitively priced products. This can be achieved only, when the bioconversion process, whereby seedling larvae and feed as input turn into mature larvae and frass as outputs, delivers a positive economic value. This study proposes a mass-balance based cost-benefit analysis model, including as-is mass balance and dry-matter mass balance, that enables a simple, practical and comparable assessment for research and industry with harmonised parameters and definitions. The key parameters proposed for the model are feedstock (weight kg, moisture%, unit cost as is $/kg), seedling larvae (weight kg, moisture%, larvae average weight g/unit, count and cost, whereby cost is normalised to $/g neonates at 50 000 larvae/g), mature larvae (weight kg, moisture%, average weight g/count, count and value/price $/kg) and frass (weight kg, moisture% and value/price $/kg). Additional parameters considered are growth area (m2), density (larvae count/m^2) and cycle time (days). Key process ratios used are bioconversion efficiency (BCE, also larvae/feed ratio), frass/feed ratio, frass/larvae ratio, larvae-out/larvae-in (LOLI) and bioconversion gross margin (BGM). The BGM is calculated by multiplying weight with unit value of the outputs and subtracting the sumproduct of the inputs; in addition unit values should be normalised to moisture content, for example if frass at 30% moisture has a value of 0.1$/kg and the process yields 40% frass moisture, the unit value is discounted accordingly to 0.86$/kg. The model was explored by analysing a selection of experimental and production data. Findings included anti-proportional relationship between average weight and density balancing a relatively stable total weight of larvae, higher BCE for higher larvae densities, trade-offs between feed weight, larvae density and dry matter losses and dry matter losses typically ranging between 30-60%. Consequently, trade-offs can be quantitatively evaluated, for example higher larvae density resulting in higher larvae yields and feed efficiencies (lower feed costs), but higher seedling costs. More feed resulting in higher larvae yields per growth unit, but also in higher dry matter losses and therefore lower frass value. The model offers assessment of multiple parameters and identification of heuristics ranges to evaluate 'what's good enough' for profitable bioconversion and demonstrates its value as a simple, comprehensive and standardised framework enabling bioconversion process evaluation and comparison for both research and industrial contexts.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Thermal effects on growth and development of *Duolandrevus* (*Eulandrevus*) *ivani*

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In 2024, a densovirus outbreak caused mass mortality in the two-spotted field cricket (*Gryllus bimaculatus*), resulting in substantial disruption to the supply chain of cricket farms in South Korea. This event highlighted the need to explore additional indigenous genetic resources that could potentially contribute to the stability of insect-based feed production systems. In this context, the present study examines the life-history traits of *Duolandrevus* (*Eulandrevus*) *ivani*, a large cricket species native to Jeju Island, under different thermal conditions, with the aim of assessing its potential applicability for industrial rearing. Growth, adult emergence, and survival of *D. ivani* were monitored at constant temperatures of 20, 25 and 30 °C. Developmental performance was quantitatively evaluated using area under the curve (AUC) analysis derived from weekly body weight measurements. At 20 °C, development was markedly delayed, resulting in a low AUC value (2.92) and a relatively small final body weight (approximately 0.30 g). In contrast, individuals reared at 25 °C and 30 °C exhibited substantially higher AUC values (10.98 and 12.32, respectively), and reached comparable maximum body weights of around 0.63 g. Adult emergence patterns varied with temperature. At 30 °C, emergence occurred earlier and within a narrower time window, whereas at 25 °C, emergence was distributed over a longer period. Adult survival appeared to be relatively consistent across temperature treatments, with mean survival times of approximately 26–27 weeks. In addition, body weight differed significantly between sexes, with females tending to be heavier than males. Overall, these results suggest that *D. ivani* can maintain stable growth and survival within the temperature range of 25–30 °C. The more synchronized emergence observed at higher temperatures may offer practical advantages for harvest management in mass-rearing systems. Although further studies on nutrition, density, and large-scale rearing performance are required, the present findings indicate that *D. ivani* could be considered a promising candidate as a supplementary or alternative feeder insect, and provide baseline information for the development of future rearing protocols.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Industry shakeout: risk signal or normal maturation phase?

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The insect industry has recently experienced increasing reports of financial distress and company failures following a period of strong capital inflow. While often interpreted as negative signals for sector viability, similar patterns have historically occurred in emerging manufacturing industries during early industrialization phases. In this work, we analysed scale-up performance, commissioning timelines, and operational stability indicators across multiple commercial insect production facilities. The objective was to evaluate whether recent financial failures reflect sector-specific weaknesses or typical industry lifecycle dynamics observed in emerging manufacturing sectors. Comparable developments were identified in other emerging technology sectors. The cultivated meat sector demonstrates that even well-funded companies may fail when technological scale-up, regulatory development, and capital market confidence do not progress at similar rates. Early solar photovoltaic manufacturing similarly experienced failures of highly funded companies despite long-term sector growth and eventual industrial maturity. These patterns are consistent with industry lifecycle models describing early experimentation phases followed by consolidation and emergence of dominant production designs. Our analysis indicates that commercial insect farming is entering a comparable phase typical for capital-intensive industries transitioning from pilot to industrial scale. Several structural drivers were identified. Capital was raised during a period characterised by low interest rates and strong investor appetite for sustainability-driven technologies, incentivizing rapid scale-up. In multiple cases, large-scale production facilities were constructed while biological and process technologies were still under development. Analysis of implemented facilities showed that scaling feedstock handling capacity from pilot levels (≈10 t/day) to industrial levels (>300–500 t/day) introduces non-linear complexity in logistics, process integration, climate control, and biological performance. Results indicate that early operational output typically reaches approx. 40–70% of designed capacity during the first operational year, while time to stable production varies significantly between sites. Furthermore, strong IP-driven strategies resulted in parallel development of proprietary technologies, limiting cross-sector knowledge sharing during early industry formation. Early market positioning strongly focused on alternative protein markets, while waste management and nutrient recovery applications were comparatively underexplored. Overall, the results demonstrate that current developments are consistent with established industry lifecycle models describing early experimentation phases followed by industry shakeout and consolidation.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Drying technologies for black soldier fly larvae: process efficiency, energy demand, and product quality

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Black soldier fly larvae (*Hermetia illucens*) (BSFL) are increasingly recognized as a sustainable source of protein and micronutrients for food and feed. Owing to their high initial moisture content, drying is a critical post-harvest step affecting product quality, energy use, and industrial feasibility. This study compares five drying technologies — sun drying (SD), hybrid solar–electric drying (HSED), vacuum freeze drying (VFD), greenhouse drying (GD) and microwave drying (MD) — to identify efficient and sustainable processing options for BSFL. Fresh larvae were dried to a final moisture content below 8% using each method. Drying performance was assessed through drying time, yield, specific electrical energy consumption (SEEC), and average drying rate (ADR). Dried larvae were further characterised for proximate composition, physicochemical and techno-functional properties, microstructure, and thermal stability. Drying time ranged from 120 h for SD to 0.33 h for MD, with intermediate durations of 6 h for HSED, 5 h for GD, and 72 h for VFD. MD achieved the highest ADR (5.16 kg water/kg dm · h), followed by GD (0.34 kg water/kg dm · h) and HSED (0.29 kg water/kg dm · h). VFD exhibited the lowest ADR (0.02 kg water/kg dm · h) but resulted in the lowest residual moisture content (3.61%). Energy demand varied substantially, with VFD requiring 206.86 kWh/kg, compared to 3.24 kWh/kg for MD and 1.62 kWh/kg for HSED, while SD and GD operated without electrical energy input. Drying method significantly influenced larval composition and functionality ($P < 0.05$). Protein content ranged from 33.36% (GD) to 44.63% (VFD), and crude fat from 24.71% to 34.80%. HSED yielded a balanced nutritional profile (42.03% protein, 32.05% fat) with a high energy value (497.57 kcal/100 g). Functional properties were highest in VFD and HSED, whereas GD showed the lowest performance. Structural analyses revealed marked tissue damage in GD and MD samples, while VFD best preserved microstructure. Overall, clear trade-offs exist between drying efficiency, energy demand, and product quality. Although VFD ensures superior quality preservation, its high energy requirement limits scalability. HSED represents a favourable compromise, combining low energy consumption, short drying time, and acceptable product quality, making it a promising option for sustainable large-scale BSFL processing.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Design of an industrial modular *Tenebrio molitor* farm for the food industry

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Eleven years after the 2015 approval of the UN Sustainable Development Goal "Zero Hunger" and the 2021 European Union (EU) legislation on insect-based food products, the commercial insect farming sector faces challenges related to innovation in systematized breeding equipment. These challenges focus on maintaining food safety standards, 24/7 operability, and ergonomic conditions for tools and machinery. This article, describe the process for designing a modular *Tenebrio molitor* breeding facility, addressing the aforementioned challenges. Based on opinions, the needs and frustrations of insect producers were identified. Additionally, through an operational breakdown, postures involving high physical effort and repetitive movements were analysed. Similarly, the optimal growth parameters for *Tenebrio molitor* were considered. With this information, a Value Proposition Canvas was integrated to define the initial design characteristics of the breeding facility. The design process was approached using the Design Thinking methodology, covering the first step, Empathize, with the conclusions from the Value Proposition Canvas. The Define problem step was complemented by a technological screening of the Mexican Institute of Industrial Property and WIPO's databases to establish design constraints and avoid including intellectually protected elements. The Ideate step was materialized through proposal sketches, selecting one for the final design in CAD software. The Prototype and Testing stages are in process and the application of the first three stages of Design Thinking led to the conceptualization of a digital prototype with an innovative design. The finalized design of the modular industrial breeding facility is characterised by:

(1) Dimensions: 50 × 40 × 160 cm.
(2) Stackable design for optimised vertical space utilisation.
(3) Mechanical system for feeding, growth and sifting featuring 8 trays.
(4) Compatible with remote visual, temperature and humidity monitoring systems.

Its primary innovation is the tray sifting system, which facilitates cleaning, separation, and feeding during larval growth, resulting in significantly reduced human effort. Discussion. The Prototyping phase is being developed with the certainty of integrating a design that meets the sector's requirements through an innovative, scalable, and industrial-grade proposal. Identified areas for improvement will be evaluated during the Testing phase to determine their inclusion or dismissal.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Friend, foe or feint? Metagenome-guided insights into *Scrofimicrobium* in black soldier fly larvae

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Larvae of the black soldier fly (*Hermetia illucens*, BSFL) are viewed as a sustainable alternative protein source due to their capacity to convert low value waste streams, such as food waste, manure, and agricultural side streams, into high value insect biomass. This broad substrate flexibility is thought to be partially mediated by the BSFL gut microbiome, which comprises diverse bacteria, fungi, and viruses that may contribute metabolic and protective functions. Previous studies have shown that the composition of the BSFL gut microbiome is shaped by environmental factors, including diet and rearing conditions, as well as host related factors, resulting in considerable variability in the insect's gut bacterial composition between studies. Nevertheless, several bacterial taxa have been repeatedly detected across independent investigations, suggesting that they may be intrinsically associated with BSFL and play a role in supporting its niche as a generalist detritivore. Our recent metagenomic analyses have identified a putative novel strain of *Scrofimicrobium*, a genus often described in association with BSFL. In the present study, we investigate the potential functional role of Scrofimicrobium_sp_concoct.67, a metagenome assembled genome (MAG) recovered from whole genome sequencing of BSFL reared on two distinct diets. As an initial step, over 600 KEGG annotated genes were examined to identify metabolic pathways that could facilitate the conversion of nutrients commonly present in BSFL substrates, as well as functions that may enhance microbial survival in the gut environment. In addition, several genes associated with antimicrobial resistance were detected, indicating a possible protective role for the bacterium within the gut microbial community. To assess whether the predicted genomic functions are biologically relevant and actively expressed, ongoing work aims to isolate the *Scrofimicrobium* strain using Actinomycetota selective agar media. Candidate isolates are being identified using two sets of primers specifically designed from the Scrofimicrobium_sp_concoct.67 MAG. Successful isolation would enable downstream phenotypic testing, including the evaluation of metabolic capabilities and antimicrobial resistance profiles using high throughput, plate based substrate utilisation assays. Collectively, this work illustrates how metagenome mining can guide the identification of potentially beneficial gut associated bacteria and outlines a workflow for linking genomic predictions to laboratory validation. In the longer term, such microorganisms may be explored as probiotic candidates to support BSFL performance on specific waste substrates.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Limits of enzymatic and chemical chitin degradation in *Tenebrio molitor* larvae

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The increasing use of insects as a sustainable protein source in livestock nutrition is constrained by the presence of chitin in the exoskeleton, which potentially impairs digestibility and nutrient availability. Efficient strategies to reduce or remove chitin are therefore essential to improve the nutritional value of insect-derived feed ingredients. This study compared different chemical, enzymatic and mechanical approaches for chitin reduction in *Tenebrio molitor* larvae. Chitin degradation was investigated using 6 approaches: enzymatic hydrolysis with chitinase, enzymatic pre-treatments with lysozyme, ultrasound or hydrogen peroxide, as well as partial and total hydrochloric acid hydrolysis. All treatments included an initial protease pre-treatment to disrupt the protein-chitin matrix. In addition, a mechanical separation process was evaluated to physically remove chitin-rich fractions during protein meal production. Chitin contents were quantified, with a limit of quantification (LOQ) of 1 g/100 g dry matter. The pre-treatments resulted in only minor or negligible chitin reduction. Partial acid hydrolysis achieved approximately 30% chitin reduction, while total acid hydrolysis exceeded 80%. In contrast, mechanical separation proved to be the most effective strategy, yielding an almost complete removal of chitin from the protein meal. Residual chitin levels were only slightly above the LOQ. The mechanically separated chitin fraction was obtained as a distinct side stream. The results confirm the high crystallinity and recalcitrance of insect chitin, which strongly limits enzymatic degradation without prior structural disruption. While acid hydrolysis was effective, its practical application is restricted by safety, environmental, and economic concerns. Mechanical separation emerged as the most efficient and scalable approach, enabling near-complete chitin removal while simultaneously generating a chitin-rich side stream suitable for further valorisation. This strategy offers a promising pathway for improving protein quality in insect-based feed ingredients and supports integrated concepts for insect biomass.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effect of water-to-larvae ratio during boiling on processing performance and quality of black soldier fly larvae

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Boiling is widely recognized as an effective post-harvest processing step in black soldier fly management. Rapid larval deactivation is a key factor influencing final product quality as it immediately stops metabolic and enzymatic activity. By quickly inactivating enzymes and reducing microbial activity, it prevents protein degradation, lipid oxidation, and off-flavour formation. Prompt larval deactivation also helps preserve nutritional value, colour, and of the final product and improves stability and process consistency. Despite extensive research on drying methods, the influence of boiling conditions remains poorly explored, although it may strongly affect heat transfer efficiency and product quality. This study investigated the effect of different water-to-larvae ratios during boiling on the quality of dried larvae, with emphasis on colour attributes. Five treatments were tested (6 replicates/treatment) using constant water volume (0.425 L) and larval loads of 0.3 (T1), 0.6 (T2), 0.9 (T3), 1.0 (T4), and 1.2 (T5) kg/l. The time required for most larvae to be deactivated (MD) and for full deactivation (FD) was assessed by two operators, showing excellent agreement (ICC>0.99). The minimum temperature (MT) reached after larval addition and the temperature at larval removal (RT) were recorded. Boiling was continued until all larvae observed at the surface were fully deactivated. Larvae were then dried at 70 °C for 18h, ground, and analysed for colour (L^*, a^*, b^*). Data were analysed by One-way ANOVA (SPSS 20.0, $p \leq 0.05$). The shortest MD and FD durations were observed in T1 and T2, while the longest in T5 ($p < 0.05$). MT was highest in T1 and T2 and decreased with increasing larval biomass ($p < 0.05$) while T3, T4, and T5 showed comparable MT values ($p > 0.05$). RT followed a similar pattern, with higher values in T1 and T2 compared to T3 and T4 ($p < 0.05$), while T5 showed intermediate values. All treatments exhibited short MD times (<40 s), although FD extended to several minutes at higher larval loads. Colour analysis indicated higher L^* values in T1 compared with T3 and T4 ($p < 0.05$), while T2 and T5 showed intermediate lightness. Only T1 exhibited lower a^* values ($p < 0.05$), whereas b^* values were higher in T1 and T5 compared with the remaining treatments. Overall, the larvae-to-water ratio significantly influenced processing temperature, deactivation dynamics, and colour attributes, highlighting the importance of ratio optimisation to preserve product quality.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Optimised bioprocessing of *Tenebrio molitor* larvae for protein and oil valorisation

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Edible insect fractionation has the potential to valorise major insect fractions while improving their quality and applicability as food ingredients. Protein concentrates/isolates with high functionality may enhance food quality and consumer acceptance. Nonetheless, developing scalable and efficient processing strategies remains a key challenge for industrial implementation. Membrane filtration, a rarely explored yet scalable and sustainable method, was first reported by our research group to recover alkali-solubilised protein from *T. molitor* larvae using a 50 kDa cut-off membrane; however, despite yielding highly pure protein fractions (>80%), the approach was limited by modest extraction yields (33%) and protein recovery rates (30%) on a defatted basis. This study aimed to optimise the complete fractionation process (defatting, solubilisation and filtration) with emphasis on process efficiency and scalability. Defatting was evaluated using mechanical pressing (screw press) and Soxhlet extraction with various solvents and their combinations. Pressing resulted in a recovered defatted flour (60.50 ± 0.00 g/100 g) with high residual fat (29.96 g/100 g) and excessive temperatures (>100 °C), which can promote protein degradation, whereas the oil (32.08 g/100 g) showed high impurity levels. Soxhlet extraction achieved effective lipid removal, showing no significant differences among solvents; still, ethanol provided the highest fat extraction efficiency (40.93 ± 1.16 g/100), yielding oils with favourable fatty acid profiles, characterised by higher polyunsaturated and lower saturated fatty acid contents. Protein recovery was subsequently optimised through alkaline solubilisation followed by ultrafiltration (UF). A Box–Behnken design (3 levels) was used to test pH (10–13), temperature (25–50 °C), time (2–4 h), and solute/solvent ratio (1/25–1/50). The pH and solvent ratio were identified as key factors. Optimal conditions were 44.0 °C, pH 12.98, ratio 1/44 and 2 h 55 min. Centrifugation conditions showed no significant effect. UF using 10- and 50-kDa membranes resulted in similar extraction yields and protein recovery rates (>10 kDa: 48%, 43%; >50 kDa: 49%, 40%, respectively). Overall, the optimisation enabled the efficient recovery of both protein and oil fractions from *T. molitor*, resulting in improved yields and product quality. Ongoing characterisation and semi-industrial-scale-up further support the potential of this approach as a sustainable, scalable strategy.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Grill or chill: preliminary results on how does temperature affect the performance of crickets?

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Microclimate temperature is among the critical factors influencing the performance of farmed insects. This study examined the effect of temperature on growth, feed conversion ratio (FCR), and nutritional value of two cricket species: the house cricket (*Acheta domesticus*) and the Jamaican field cricket (*Gryllus assimilis*). The experiment was conducted in climate chambers under controlled conditions (65% relative humidity and a 12:12 photoperiod) at four temperatures (25, 28, 31 and 34 °C), with chicken feed used as the diet. The trial started with 0.25 g of one-day-old pinheads and was terminated after 40 days. Regardless of temperature, house crickets achieved higher yields with similar FCR as Jamaican field crickets. Temperature significantly influenced most of the monitored parameters. In house cricket, harvested biomass increased with increasing temperature, whereas differences in yields among Jamaican field cricket groups were not significant. FCR and frass production increased with temperature in both species. Significant differences in chemical composition were also observed. In Jamaican field cricket, protein content decreased while lipid content increased with rising temperature. In AD, protein and lipid contents did not differ significantly among groups reared at 25–31 °C; however, crickets reared at 34 °C contained more protein and less fat than those reared at lower temperatures. This study highlights the importance of temperature in cricket farming and emphasizes the need to balance input costs with output gains. The two species exhibited distinct responses to temperature. Overall, the house crickets appears more suitable for insect farming, as higher rearing temperatures increased biomass yield, albeit at the cost of higher feed conversion rates. Further research should focus on a more detailed analysis of nutrient quality, verification at a larger scale, and evaluation using different diets. The study was funded by INTER-COST project titled “A comprehensive strategy for the safe production of nutritious edible insects” nr. LUC25019.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

From substrates to safety: larval production and HACCP-based risk analysis of *Hermetia illucens* meal

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This study examined how alternative feeding substrates, including pig manure, influence the productive performance of black soldier fly (*Hermetia illucens*) larvae and the associated risks during meal production under a Hazard Analysis and Critical Control Points (HACCP) approach. Larvae were reared on two experimental substrates containing 20% and 25% pig manure and a control diet. Development time, biomass production, dry matter yield, feed conversion efficiency, nitrogen-adjusted and corrected feed conversion efficiencies, bioconversion rate and residue reduction were measured. The diet containing 25% pig manure produced the best overall performance, reaching harvest at 19 days and yielding 1446 ± 53.4 g biomass and 428 ± 25 g dry matter. Larvae from all treatments were processed into meal using a laboratory-developed protocol, informed by the literature and optimised through pilot trials to preserve product quality. Proximate composition was broadly similar among treatments, except for moisture, ash and nitrogen-free extract. Protein content ranged from 40.5 ± 1.79 to 41.0 ± 0.57%, while ether extract ranged from 26.7 ± 0.9 to 32.6 ± 0.4%. Amino acid profiles were stable, whereas fatty acid composition showed high lauric acid levels (32.5 ± 9.69 to 45.0 ± 0.46%). Calcium, phosphorus and iron were prominent in mineral profiles, and functional properties such as water-holding capacity and oil absorption supported technological performance in feed formulations. Microbiological analyses met safety criteria, except for the aerobic mesophilic count, which exceeded values reported for processed BSF larvae in previous studies. This count slightly surpassed the reference hygiene threshold ($<1 \times 10^4$ CFU g^{-1}) recommended by the IPIFF for insect-derived feed ingredients. This result highlights the need to reinforce Good Manufacturing Practices (GMP) during handling and processing. A HACCP-based analysis of the production process identified biological, chemical and physical hazards, including microorganisms, contaminants, allergens and particulate matter. Two Critical Control Points were defined: raw material reception to manage chemical risks and drying to control biological risks. Additional hazards were addressed through process controls, with monitoring variables, critical limits and corrective actions established. Taken together, these results show that substrate composition shapes larval performance and that HACCP provides a robust framework for identifying hazards and strengthening process control in *H. illucens* meal production for animal feed.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Why dry matter is not enough: comprehensive physical property characterisation of black soldier fly larvae substrates

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Black soldier fly larvae (BSFL) feed and develop in biowaste substrates whose physical properties can be as critical as nutrient content for stable and efficient production. Across diverse biowastes, key properties such as bulk density, water-holding capacity (WHC), and particle size vary widely, influencing aeration, microbial activity, heat generation, and overall bioconversion efficiency. Despite their importance, these parameters remain poorly characterised. To close this knowledge gap, we performed the first comprehensive physical property characterisation study of BSFL substrates to establish reference values, identify correlations, and enable substrate comparison. We analysed 16 commercially relevant BSFL substrates (i.e. mixtures of individual biowaste streams) with differing compositions provided by nine companies. Analyses included bulk density, viscosity, penetration force, WHC, water release by filtration (WRF), free water, and dry matter (DM). Relationships among structural, mechanical, and water-related properties were examined using Pearson correlations and principal component analysis (PCA) in Rstudio. Although all substrates were optimised for efficient bioconversion, their physical properties differed substantially. For instance, bulk density varied over a twofold range (0.5–1.1 g cm^{-3}), viscosity spanned more than two orders of magnitude (8–2000 Pa · s at shear rate 1), and penetration force differed by over 300-fold (0.01–3.30 N). Likewise, WHC ranged from 2–5 g water g^{-1} DM (a 150% difference), WRF from 0–16%, free water from 0–10% of total water, and DM content from 24–33%. Strong statistically significant internal coupling was observed within groups of similar properties: mechanical properties (viscosity and penetration force) showed a strong positive correlation ($r = 0.95$), and water-release metrics (WRF and free water) were likewise closely linked ($r = 0.85$). In contrast, bulk density showed weak relationships with other properties, indicating that substrate structure represents an independent physical characteristic. PCA confirmed that BSFL substrates vary across multiple, partially independent physical dimensions, demonstrating that DM — or any single factor alone — is insufficient to capture the physical complexity of BSFL feeding substrates. This study supports the need to consider physical substrate properties as design and control parameters in BSFL production and, while controlled rearing trials were not feasible due to company restrictions and multidimensional substrate variability, it provides a foundation for future work linking substrate physical properties to bioconversion performance.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Antioxidant activity of edible insects: effect of method of extraction, bioactivity assay and diet

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Edible insects are increasingly studied for their antioxidant activity, which is influenced by species and diet. However, the subsequent production of insect-based ingredients for health, such as antioxidant extracts, will also depend on the extraction method. Therefore, this study compared the antioxidant activity of extracts from *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) larvae obtained by two extraction methods typically used to produce bioactive extracts from natural sources, such as ultrasound-assisted (UAE) and pressurized liquid extraction (PLE). For both methods, the effect of diet was also tested by comparing antioxidant extracts obtained from larvae fed conventional diet or the by-product olive pomace. Both species were reared on their conventional diet (wheat bran) or wheat bran including 50 % of olive pomace (OP). Reared larvae were slaughtered, dried, ground, and defatted, and extracted by UAE (with EtOH 70%, ratio sample to solvent 1:20, for 25 min at 40 °C) and PLE (with EtOH 70%, ratio sample to solvent 1:20, 10 min, at 80 and 120 °C). Antioxidant activity was evaluated by the two popular DPPH and ABTS radical scavenging assays. Results showed that extraction method and diet markedly influenced antioxidant activity. It was also evidenced that the antioxidant assay used was relevant. In general, UAE extracts showed higher antioxidant activity than PLE extracts for DPPH (46% vs 44.9%), whereas ABTS results were similar between both extraction methods. TM extracts exhibited greater DPPH inhibition (53.7%), whereas HI extracts showed higher ABTS inhibition (55.9%). OP feeding enhanced DPPH inhibition in both species (up to 30%), while ABTS activity appeared similar between diets. For PLE, higher temperatures (120 °C) increased DPPH inhibition in both species and diets, reaching the highest for TM-OP (44.5%), whereas ABTS inhibition was favoured at 80 °C, mainly for HI-OP (55.9%). In conclusion, antioxidant activity of insect extracts is influenced by species, diet, extraction method, and antioxidant assay used. These findings evidence the potential selective production of antioxidant insect extracts through specific extraction strategies, together with the valorisation of agri-food by-products, to produce ingredients for health-related applications. Acknowledgements: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

The influence of disturbance of *Hermetia illucens* pupae on egg production

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Egg production is a key indicator in black soldier fly (*Hermetia illucens*, BSF) breeding systems, as it determines the supply of progeny and the proportion of larvae that can be allocated to biomass production. Although adult reproductive performance is often optimised through cage design and environmental control, the influence of pre-imaginal handling on subsequent egg yield remains insufficiently understood. Producers may remove frass from (pre)pupae by sieving, an action that consists of high-frequency micro-impacts. This intervention improves the accuracy of estimating the desired number of flies per cage, but it may also impose mechanical disturbance that affects metamorphosis, adult fitness, and ultimately reproductive output. Therefore, 3 trials were performed to evaluate the impact of vibrations (sieving) on egg production. Each trial consisted of 24 reproduction cages (60 × 60 × 90 cm), maintained under uniform conditions (30 °C, 80% RH) with controlled lighting, water access, lures and egg traps. Eggs were collected at fixed two-day intervals until production ceased, and total egg yield (g) per cage was used as the response variable. In the first trial, pupae (± 250 g) were subjected to a harsh treatment of daily sieving (1 minute) or no sieving at all. This resulted in a 97% reduction in egg production, which prompted a closer examination of the intensity of the mechanical disturbance. A second trial was therefore designed in which 24 batches of late prepupae were prepared. Each day, 3 batches were randomly selected for a single one-minute, maximum-intensity sieving treatment, ensuring that each batch was disturbed only once and allowing the identification of the most sensitive developmental days prior to adult emergence. The results indicate that sieving when prepupae or young pupae (1–3 days old) are present has no significant negative effect on egg production. Nevertheless, a single one-minute sieving event 4 days into pupation (or older) decreased egg output by 37%. A final trial quantified the relationship between sieving intensity and reproductive performance using an accelerometer to measure the amplitude of both hand-sieving and mechanical-sieving motions. Mechanical sieving within a certain low-amplitude range did not negatively affect egg yield, whereas exceeding this safe threshold led to a linear decrease in egg production with increasing amplitude. No effect on pupae eclosion was observed. In our setup, this threshold corresponded to accelerometer readings averaging 100 G. This leads us to conclude that BSF pupae are more fragile than expected. Handling pupae is therefore best avoided and, when necessary, should be performed as early as possible and with great care to prevent reductions in egg production.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Slaughtering methods outcomes on the oxidative stability, antioxidant response, safety and functionality of *T. molitor*

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A strong focus has been placed on safety, pre-processing, functionality, and the fractionation of insects as food ingredients to enhance product quality and consumer acceptance. However, the impact of insect-slaughtering processes on various properties remains poorly understood. To address this, reared *T. molitor* larvae, collected at 8-9 weeks old, were subjected to 3 slaughter methods, each in 2 different conditions: Freezing at –24 °C for 3 h (FZ-1) and 24 h (FZ-2); Blast-Chilling at –45 °C, for 3 h (BC-1) and 1.5 h (BC-2); and Blanching at 100 °C for 5 min without (BL-1) and with a 0 °C (5 min) pre-chilling step (BL-2). The techno-functional properties, oil oxidative stability, antioxidant enzyme activity, and microbiological safety were evaluated. Foaming (8–12%) and emulsifying (2–4%) capacities and oil (8.5–9.9%) and water (7.6-12.5%) binding capacities showed no significant differences among treatments due to high lipid and chitin content in the whole insect flour. In contrast, the processing methods exerted a pronounced impact on colour. The BC-1 yields the highest lightness value (L^* = 35.20 ± 0.76), whereas BC-2 results in the most severe browning index (47.78 ± 4.89). Regarding microbial load, evaluated upon sample processing and after 6 months of storage in sealed bags (c.a. 25 °C), slaughtering methods had a significant effect on TVC and Enterobacteriaceae, with lower loads for BC methods. However, all samples stayed within EU safety limits (EFSA). FZ-2 presented the lowest lipid oxidation markers in oil samples (3.76 ± 0.71 mg KOH/g, 0.24 ± 0.15 mg MDA/kg, and 1.00 ± 0.00 meq O_2/kg oil) and minimal antioxidant enzyme activity (SOD 0.01 ± 0.01 U/mg, CAT 2.73 ± 0.01 U/mg and GPx 0.97 ± 0.14 U/mg), indicating preserved oxidative stability. By contrast, BL-2 induced pronounced lipid oxidation, reflected by elevated peroxide value (71.21 ± 1.21 meq O_2/kg oil) and TBARS (2.57 ± 0.20 mg MDA/kg), accompanied by increased CAT (15.21 ± 0.28 U/mg) and GPx (3.59 ± 0.00 U/mg) enzyme activities. BC samples showed intermediate levels of oxidative damage, suggesting that rapid cooling may promote oxidative stress by disrupting cells. Overall, these results show that slaughtering strategies modulate oxidative stress in *T. molitor* larvae, thereby influencing lipid stability and antioxidant enzyme activity, without affecting the techno-functional properties of the flours.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Induced growth suspension for enhanced logistics in black soldier fly production: industrial validation of the StayPrime

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The insect protein is affected by several bottlenecks, such as high production costs, high complexity and natural fluctuations in egg output. These affect the entire value chain, from full-life cycle producers to specialized breeders, to small farms and R&D labs. This study evaluates StayPrime, a patented technology that reduces production costs and logistical complexity by inducing suspended animation to extend the shelf life, storage, and transportability of black soldier fly (BSF) neonates and juveniles. StayPrime was first evaluated through trying out sample tests at different producers, successfully suspending the young larvae for 7–14 days. These tests were followed by a one-year validation phase, during which suspended BSF juveniles were shipped and stored across multiple insect farms. This external validation ensures that the technology is robust and transferable across different operational environments. In the validation phase, batches of juveniles were packaged with StayPrime and shipped to regional rearing partners for 7-day storage before being reared in commercial settings. Performance was benchmarked against non-arrested controls to evaluate survival, weight and Feed Conversion Ratios (FCR) in production. Findings confirm that StayPrime successfully arrests development with minimal weight flux and high post-suspension survival (>80%). During the trying out tests, suspended neonates exhibited a significant compensatory growth effect, reducing overall production time from 17 to 14 days and increasing final larval weight from 130 mg to 151 mg compared to the control. The one-year validation confirmed that StayPrime-treated juveniles consistently averaged high harvest survival (>90%), with mean weights of 128–136 mg and mean FCRs of 1.35–1.39 (calculated as kg dry matter feed per kg fresh larval weight), matching standard production benchmarks throughout the period. Economically, the technology reduced shipping costs by more than 50% by replacing multiple express deliveries to different farms with a single weekly shipment under standard condition. StayPrime provides a robust applicable solution for stabilizing the insect supply chain and lowering the barrier of production costs. By creating "buffer capacity" for neonates or juveniles, the technology offers immediate value to researchers, pilot-scale innovators, and full-scale industrial producers alike. To date, StayPrime has facilitated the production of over 100 tonnes of BSF biomass across diverse partner facilities. A standardized physical product is currently under development and is expected to be available on the market soon. This commercial solution offers a plug-and-play tool to streamline production schedules, reduce overhead, and enhance efficiency across the global insect farming industry.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Hatching methods black soldier fly eggs: implications for industrial rearing efficiency

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The black soldier fly (BSF) is a key species in circular economy models, where early developmental stages strongly influence production efficiency. Although most rearing protocols rely on moist substrates for egg hatching, BSF eggs can hatch without nutritive support. This study compared two hatching methods under controlled conditions to optimise egg-to-larvae conversion at industrial scale. Eggs (<24 h old) from an in-house colony were collected using standardized 3D-printed oviposition devices (Kinsect S.r.l), weighed, and incubated in plastic containers (20 × 30 cm) using either "wet hatching" (WH; Gainesville diet, 70% water) or "dry hatching" (DH; non-nutritive support). Experiments were conducted at 27 °C and 70% relative humidity with ten replicates per treatment. In WH, containers with fresh wet substrate beneath the oviposition platforms were replaced daily from first hatch to day 5, and larvae dropping into the substrate were counted daily. In DH, neonates dropping onto the container surface were collected daily, weighed, and transferred to WH substrate to estimate larval counts. Day-specific counts reflected larval recovery integrating hatching timing with early survival and handling effects rather than representing pure hatching rates. Larval output was expressed per gram of eggs, and survival was calculated using a literature-based egg weight (0.028 mg). Total larval output, survival and day-specific counts were compared between treatments using independent samples *t*-tests. Total larval output and survival did not differ significantly (WH: 20 075 ± 4531 larvae (g eggs)$^{-1}$, 56 ± 13%; DH: 18 053 ± 4301 larvae (g eggs)$^{-1}$, 51 ± 11%). Temporal patterns differed: WH showed earlier larval recovery on day 3 (7783 ± 3289 larvae), whereas DH had no larvae. By day 4, DH surpassed WH in larval recovery (16 844 ± 4356 vs. 11 920 ± 5710 larvae), and on day 5, DH still yielded higher counts (1208 ± 583 vs. 372 ± 316 larvae), with no larvae observed thereafter. Although cumulative output was similar, dry hatching concentrated larval emergence, reduced contamination risk, and allowed flexible handling. These results suggest that dry hatching can be implemented in industrial BSF production to synchronize larval availability and improve operational efficiency, while minimizing challenges related to early larval mortality, uneven distribution, and substrate contamination.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effects of dietary energy and protein levels on growth performance and chemical composition of *Tenebrio molitor* larvae

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To optimise protein production from insect larvae, a comprehensive understanding of their nutritional requirements, production performance, and the chemical composition of the produced biomass is required. In this study, nine experimental diets based on wheat bran were formulated, with corn, soybean meal, and sugar beet pulp incorporated to modulate dietary energy and protein levels. Three proteins and three energy levels were combined. Each of nine treatments had 4 replicates, and each replicate contained 2 g of *Tenebrio molitor* larvae. The results showed that larval growth performance was significantly affected by protein level in diets, whereas energy level had no significant effect on weight gain expressed as grams per day per tray. Weight gain ranged from 0.57 g/day per tray for larvae fed low-energy, low-protein diets to 0.71 g/day per tray for those receiving high-protein, medium-energy diets. FCR varied between 1.83 and 2.34 and was significantly influenced by both energy and protein levels in the diet. The protein effect on FCR was significant in four of the five weeks of the duration of the trial and in the whole period. Lipid content ranged from 38.5% in larvae fed low-protein diets to 26.9% in those fed high-protein diets. These results indicate that dietary protein level is a key driver of growth performance, while both energy and protein levels strongly influence feed efficiency and body composition. From the standpoint of feed cost per unit of gain, medium levels performed best. Low protein resulted in reduced gains, while high protein did not lead to sufficient improvement compared to medium levels to justify the increased cost. Gain was only 3% higher, with price 7% higher for the high protein feed compared to the medium protein feed. Feed cost per unit of protein gain was again affected by feed protein level, rather than energy level. The difference between medium and high protein feeds was moderate, while the low protein group performed worse. Despite better protein conversion when low protein feed is used, the cheapest protein production is achieved with higher protein levels. The findings highlight the importance of diet formulation as a practical tool for steering both production efficiency and the nutritional quality of insect biomass intended for use as a sustainable protein source. To obtain sustainable insect farming, this emerging industry will have to focus on the economic parameters of production. This research shows that finding an economically optimal solution might be a challenging task involving integrating multiple important parameters.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

DIC technology on the BSF larvae preservation: microbiological decontamination, quality characteristics and COVs profile

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The global population is steadily increasing, resulting in a growing demand for protein for both human and animal consumption. Edible insects represent a promising and environmentally sustainable alternative for future food systems. To make insects suitable for food or feed formulations, various processing techniques must be employed while ensuring the safety, nutritional value, and sensory quality of the final product. These techniques include conventional methods such as aqueous processing, salt treatments, solvent, detergent, alkali, and thermo-mechanical processing, as well as innovative techniques considered as green extraction or intensification methods, including enzyme, ultrasound, High Pressure (HP), pulsed electric field, and instant controlled pressure drop (DIC). Among this emerging process, DIC emerges as a relevant technology for insect processing, as it allows simultaneous microbial decontamination and modification of the physical structure of insect matrices. This study aimed to assess the effect of DIC treatment on the microbial decontamination of black soldier fly larvae, while also evaluating its role in preserving product quality and improving the sensory attributes of the resulting larvae flour. Using HS-SPME/GC–MS quantification, the volatile profile of raw dried larvae was quantitatively characterised. A total of 44 major volatile organic compounds (VOCs) were identified, including 5 acids, 6 alcohols, 7 aldehydes, 8 alkanes, 7 esters, 3 pyrazines, 5 ketones and 3 other compounds. Compared with raw BSF larvae, DIC-treated larvae flour exhibited a significant reduction in the overall volatile compound content. A short DIC treatment of 55 sec at 0.6 MPa resulted in reductions of 87.52, 64.62, 34.18 and 74.57% in the concentrations of 1,4-dimethylcyclooctane, hexanal, dodecanoic acid and acetic acid, respectively. In addition to preserving insect biomass quality (Protein and Oil), the effect of DIC processing can be attributed to the inactivation of oxidative enzymes in the larvae flour. In the other hand, some conditions of this treatment result in a markedly reduced microbial load, with low viable counts compared to untreated larvae. The application of rapid (55 s) DIC treatment at 0.6 MPa allowed the microbial reduction of 2.69 ± 0.09 log cfu/g “total aerobic count”, highlighting the effectiveness of DIC as green process. Moreover, DIC treatment was proved to be much more effective than conventional thermal processes, including water blanching, conventional steam treatment, and HP processing.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Hatching methods of black soldier fly eggs: implications for industrial rearing efficiency

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The black soldier fly (BSF) is a key species in circular economy models, where early developmental stages strongly influence production efficiency. Although most rearing protocols rely on moist substrates for egg hatching, BSF eggs can hatch without nutritive support. This study compared two hatching methods under controlled conditions to optimise egg-to-larvae conversion at industrial scale. Eggs (<24 h old) from an in-house colony were collected using standardized 3D-printed oviposition devices (Kinsect S.r.l), weighed, and incubated in plastic containers (20 × 30 cm) using either "wet hatching" (WH; Gainesville diet, 70% water) or "dry hatching" (DH; non-nutritive support). Experiments were conducted at 27 °C and 70% relative humidity with ten replicates per treatment. In WH, containers with fresh wet substrate beneath the oviposition platforms were replaced daily from first hatch to day 5, and larvae dropping into the substrate were counted daily. In DH, neonates dropping onto the container surface were collected daily, weighed, and transferred to WH substrate to estimate larval counts. Day-specific counts reflected larval recovery integrating hatching timing with early survival and handling effects rather than representing pure hatching rates. Larval output was expressed per gram of eggs, and survival was calculated using a literature-based egg weight (0.028 mg). Total larval output, survival and day-specific counts were compared between treatments using independent samples t-tests Total larval output and survival did not differ significantly (WH: 20 075 ± 4 531 larvae (g eggs)$^{-1}$, 56 ± 13%; DH: 18 053 ± 4301 larvae (g eggs)$^{-1}$, 51 ± 11%). Temporal patterns differed: WH showed earlier larval recovery on day 3 (7783 ± 3289 larvae), whereas DH had no larvae. By day 4, DH surpassed WH in larval recovery (16 844 ± 4356 vs. 11 920 ± 5710 larvae), and on day 5, DH still yielded higher counts (1208 ± 583 vs. 372 ± 316 larvae), with no larvae observed thereafter. Although cumulative output was similar, dry hatching concentrated larval emergence, reduced contamination risk, and allowed flexible handling. These results suggest that dry hatching can be implemented in industrial BSF production to synchronize larval availability and improve operational efficiency, while minimising challenges related to early larval mortality, uneven distribution, and substrate contamination.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effect of hybrid solar–electric drying temperature on the quality and functional properties of black soldier fly larvae

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Black soldier fly (*Hermetia illucens*) larvae (BSFL) represent a promising sustainable nutrient source for food and feed. Drying is essential to ensure microbial safety and shelf stability, but conventional technologies are often energy-intensive and poorly suited to small-scale or resource-limited systems. Hybrid solar–electric drying (HSED) offers a low-energy alternative, particularly in regions with high solar availability, by reducing reliance on electricity or fuel. However, its effects on BSFL quality remain poorly documented. Whole larvae were killed by freezing (–20 °C for 24 h) and dried using HSED at 40, 50, 60 and 70 °C until constant weight was reached, corresponding to drying times of approx. 25, 19, 8 and 3 h, respectively. Dried larvae were analysed for proximate composition, mineral content, pH, colour, bulk density, techno-functional properties (water and oil binding, emulsifying and foaming capacities), microstructure, thermal stability, and molecular structure. Drying temperature significantly influenced larval quality. Protein content decreased from 45.8% at 40 °C to 37.7% at 70 °C, while crude fat declined from 31.8% to 24.3%. By contrast, carbohydrate content increased from 7.3 to 21.2%, and ash from 7.1 to 9.5%. Dried larvae showed high mineral levels, notably calcium (3208–3238 mg/100 g), iron (52–53 mg/100 g), and zinc (20–27 mg/100 g). Higher temperatures resulted in darker colouration (ΔE up to 12.2), increased bulk density (0.5–0.7 g/ml), and reduced techno-functional properties, including water-binding capacity (88.2 to 74.3%) and oil-binding capacity (98.6 to 75.4%). Scanning electron microscopy revealed greater tissue damage and porosity at higher temperatures, while thermal analysis indicated high thermal stability across treatments. Overall, HSED can produce nutritionally valuable BSFL ingredients. Lower temperatures (40–50 °C) better preserve protein and functional properties, whereas higher temperatures (60–70 °C) may provide practical drying efficiency depending on the intended application.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Identification of process phases in black soldier fly larvae bioconversion of food waste

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Industrial black soldier fly larvae (BSFL) bioconversion with food waste can be hampered by challenges such as extensive heat production, reduced larval growth and variable gaseous emissions. These stem from the complex biology of the system, lacking an established succession framework. This study performed a systematic multi-omic bioconversion description via delineating process phases through the integration of macro-variables (larval mass, temperature, moisture, pH), microbial actors (fungi, bacteria) and metabolites. For industrial relevance, feeding trials were conducted in large-scale crates (40 × 60 cm) with 3 distinct food waste batches under high and low temperature regimes, with and without larvae. Throughout bioconversion, macro-variables, microbes (amplicon sequencing), metabolites (LC-MS), and VOCs (GC-MS) were tracked. We identified four consecutive core process phases via hierarchical clustering of microbes, metabolites and macro-variables. Phase characterisation employed linear mixed models, alongside ecological-role annotations of microbes. Strikingly, in the first two phases, bacteria and metabolites did not differentiate based on larval presence indicating that early metabolic shifts were driven primarily by microbes. While fermenters, constituted ≥60% relative abundance throughout the first three phases they dropped ≤40% within phase 4, alongside reductions in fermentation indicators (e.g. *Clostridium*, succinate). This drop suggested an anaerobic–aerobic shift in the substrate related to reduced moisture (–6%) and higher temperatures (+8 °C). Additionally, significant increases in antimicrobial metabolites (e.g. macrolactams, aminoglycosides) indicated intense microbial competition. Finally, PLS-DA based phase prediction via VOC patterns showed high accuracy (>80%) for phases 1,3 and 4, demonstrating the potential for real-time phase monitoring via exhaust gases. This research emphasizes the heavy influence of metabolic shifts in the substrate related to thermal peaks and emissions and suggests a phase-based framework for systematic optimisation of the process.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Microbiological food safety hazards related to *Bacillus cereus* group in black soldier fly (*Hermetia illucens*) production

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The production of black soldier fly larvae (BSFL, *Hermetia illucens*) commonly relies on agrifood by-products and organic waste streams, enabling their valorisation into high-value biomass for food and feed applications. However, these substrates may harbour microbiological hazards like foodborne pathogens. Among these, the *Bacillus cereus* group represents a major concern in insect production systems due to its endospore-forming capacity and relevance to food safety. This study evaluated the occurrence and dynamics of *B. cereus* in BSFL production by addressing two key aspects. First, the prevalence of *B. cereus* was assessed in an industrial organic waste stream (swill; i.e. supermarket food leftovers) obtained from a local waste processor and sampled at multiple time points. The effects of substrate heat treatment (60 °C, 30 min) and 7-day storage, either refrigerated or at room temperature, on *B. cereus* persistence were investigated as well. Second, pathogen survival and transmission during BSFL production on swill were assessed via challenge tests. BSFL were reared on artificial swill (a controlled substrate mimicking industrial swill, consisting of fruits, vegetables and dairy) inoculated with *B. cereus* and both vegetative cell and endospore counts were monitored. Results indicate that *B. cereus* can frequently be detected in industrial swill, with counts ranging from 6.3×10^3 to 2.5×10^5 cfu/g, occasionally reaching levels of food safety concern. Heat treatment reduced *B. cereus* counts by approx. 1.5 log cycles, leaving predominantly endospores at levels of up to 5.0×10^3 cfu/g. During storage, only vegetative *B. cereus* counts were slightly reduced in amount under both refrigerated and ambient conditions. In the challenge tests, *B. cereus* was successfully introduced into the artificial swill, mainly in its vegetative form. Preliminary results indicate a decrease in pathogen levels over time during BSFL rearing. The persistence of *B. cereus*, particularly in its endospore form, highlights the resilience of this pathogen in (potential) BSF substrates. The observed reduction during larval rearing suggests that rearing conditions may limit pathogen survival or transmission. Further experiments will assess the risks associated with inoculation of *B. cereus* in its endospore form. In conclusion, this study confirms that *B. cereus* is a relevant microbiological hazard in BSF substrates such as swill, occurring both as vegetative cells and endospores. While BSF rearing conditions may reduce pathogen levels, further research is needed to fully understand the food safety implications and to optimise risk mitigation strategies in insect production systems.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effects of long-term storage on *Hermetia illucens* larval performance

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The production of black soldier fly larvae (BSFL, *Hermetia illucens*) is widely used for organic waste conversion and as a sustainable biomass source, particularly for animal feed. As production systems scale up, maintaining reliable larval stocks becomes essential. Larval storage is often applied to manage fluctuations in production and demand, yet its long-term biological and genetic effects remain poorly understood. In this study, 7-day-old larvae (7 DOL) were subjected to repeated storage cycles at 19 °C using hydrated straw pellets sealed with aluminium foil to maintain moisture. Each cycle consisted of two months of storage followed by one generation of rearing before returning larvae to storage. By the time of this study, the stock population had undergone 32 consecutive storage cycles. Both the stock line and a continuously reared control line originated from the same initial population. To assess the impact of repeated storage, both lines were reared under identical conditions on three nutritionally distinct diets, with 100 larvae per replicate in triplicate. Survival, development time, growth curves, final larval weight, feed conversion ratio, and waste conversion efficiency were recorded. Preliminary observations from previous experiments indicate that larval survival during storage ranges from 77 to 99%, while pupal hatching ratios remain high (84–100 %), suggesting that storage does not critically impair pupal development. Slower growth has been observed in stored populations compared to continuously reared larvae. Genetic divergence between the lines will be assessed using Illumina paired-end whole-genome sequencing to determine whether repeated storage induces measurable genetic changes. By combining phenotypic and genomic analyses, this study clarifies whether prolonged storage leads to measurable biological or genetic divergence, providing insights for more robust stock management in BSFL production.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Exploring the impact of dietary changes during black soldier fly rearing on their microbiome composition and performance

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Black soldier flies are key organisms for large-scale insect rearing and bioconversion, owing to their ability to convert diverse organic substrates into high-quality biomass. Achieving true circularity, requires optimising rearing practices when nutritionally poor, low-cost substrates are used. In many systems, larvae are first raised on a nutrient-rich nursery diet before being transferred to a fattening diet. A key question is whether this two-phase feeding strategy improves performance or whether similar results can be obtained when larvae are reared directly on the fattening diet. This directly concerns dietary flexibility and how it can be used most effectively in practice. To explore the effects of dietary shifts on larval performance and the microbiome, this study investigated the impact of a diet change between the nursery stage (0 – 7 days after egg harvest; DAH) and the fattening stage (8 – 15 DAH), using chicken feed (CF) and artificial supermarket waste (SW). At the end of the nursery stage, CF-nursed larvae were more than twice as heavy as SW-nursed larvae (0.0185 ± 0.0028 g vs. 0.0074 ± 0.0009 g). After diet shift, however this effect diminished by the end of the fattening phase, though the rate at which was variable between diets. This indicates that a diet transition might results in a temporary slowdown, but not necessarily a lasting reduction in performance. Microbiome analyses based on 16S rRNA gene sequencing revealed distinct bacterial communities associated with CF-based and SW-based rearing. The CF-to-CF group consistently maintained a diverse community, whereas the SW-to-SW group showed persistently low diversity and, from DAH 9 onward, microbiome was dominated by five zero-radius operational taxonomic units. Three of these belonged to the family Lactobacillaceae, and two were identified as *Lactobacillus*. Following the diet change, the microbiome gradually shifted from a nursery-driven towards a fattening-driven profile, although full convergence was not achieved. This indicates that early-life diet leaves a lasting imprint on microbial assembly and limits later microbiome adjustment. Together, these findings show that diet – microbiome – insect interactions shape larval performance and highlight the need to consider these dynamics in multi-diet rearing protocols, as early-life nutrition can constrain later microbiome adjustment and affect responses to subsequent feeds.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Moisture optimisation for adult beetles: assessing hydration needs in *Tenebrio molitor*

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Previous studies have shown that giving a source of moisture to mealworm larvae is necessary to ensure efficient and rapid growth. In contrast, the hydration requirements of adult beetles remain poorly documented, despite indications from pilot scale production that moisture availability may strongly influence adult health and reproductive output. However, supplying moisture increases labour demands and may involve work on holidays/weekends. A better understanding of how long beetles can be deprived of moisture without compromising performance is therefore essential for efficient mealworm farming. Two dehydration–rehydration experiments were conducted to quantify the effects of moisture deprivation on adult survival, body weight and fertility (larvae/female per day). In the first experiment, five groups were created where beetles were deprived of moisture for 0, 1, 2, 3 or 4 days. Each group was replicated five times with a 100 beetles per replicate (14 days, 50/50 sex ratio assumption). Mortality and weight change of beetles was assessed over the course of 10 days to cover both the de- and rehydration period. In a second experiment, a similar set-up was used but with 0, 1, 2, 4 and 6 days without moisture. Besides the weight and survival, the daily fertility was also determined. Both experiments were performed at 27 °C and a relative humidity of 60%, wheatbran as dry feed and an agar cube as moisture source if appropriate. Across all treatments, mortality remained unaffected, even after six days without moisture. The animals did lose a consistent 5 mg (or 4%) of bodyweight per day without access to moisture for up to at least six days. The beetles fully regained their initial weight within 24 hours once moisture was reintroduced. Fertility did however does not follow this pattern. A single day without access to a moisture source did not affect fertility compared to the control. Starting from two days a reduction is visible (20-30%) with severe effects after 4 (75% reduction) and 6 days (90% reduction). More importantly, there is no immediate recovery. With 2, 3 or 4 days needed to return to control fertility levels after respectively 2, 4 or 6 days without moisture. These findings demonstrate that while adult beetles tolerate short term dehydration, their reproductive performance is sensitive to moisture availability. For example, omitting moisture provision during the weekend can reduce fertility by 20% during the weekend and, crucially, also on Monday due to the lag in recovery. Hence avoid more than two consecutive days without moisture in the breeding units.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Bodybuilding babies – an optimised Sofia Diet for black solider fly neonates

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The larvae of black soldier flies (*Hermetia illucens*; BSFL) show great promise as a high-quality source of protein. Until recently, comparing studies on BSFL on a dietary basis has been difficult. There is a need for the development of an artificial, globally available, and consistent diet that will enhance comparison effectively between studies. This study expanded the use of the artificial Sofia Diet by optimising the composition for neonates during the 0 to 5 day growing period. A series of experiments using mixture design models was used to narrow the region for optimisation. Quadratic models were used to evaluate the effect of ingredient inclusions on life history traits, with fitness (percent survival to adulthood*fecundity) and larval weight at 5 days used as key performance indicators of a successful diet. The optimal diet for neonates consisted of 0.25% xanthan gum, 75% water, 21% hydrolysed yeast protein, 3.65% sucrose and 0.1% premix, which differed from the Sofia Diet for the 5–12 day growing period. With this ingredient mixture, the model predicted 5-day-old larval weight of 22.9 mg with a biological fitness of 1098. Known nutrient requirements for BSFL were exceeded by the optimal diet and with a protein to sucrose ratio of 5.75:1, this suggested that nutritional requirements differ between neonates and fattening larvae. Analysis of ingredient effects revealed that physical properties of the diet were critical in determining larval survival. Now that an artificial and standardized Sofia Diet has been established across the larval life cycle and can sustain multiple generations, the Sofia Diet can reduce variation between studies under controlled conditions. This allows for standardisation and shared practice, enabling more reliable comparisons and enhancing collaboration among the research, academia and industry sectors.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

A framework for assessing and tracking digital maturity in the insect industry

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The insect industry is under increasing economic pressure while simultaneously being expected to improve efficiency, scalability, and sustainability. Although digital technologies and artificial intelligence are frequently discussed as potential solutions, there is currently no structured framework for systematically assessing the digital maturity of insect production systems. This study develops and applies a research framework to evaluate the level of digitalization in insect farming, with a focus on black soldier fly (BSF) production systems. The framework combines (i) industry and market analysis, (ii) an empirical survey among insect production stakeholders, and (iii) an analytical synthesis including digital maturity assessment and cross-industry benchmarking with digitalization approaches from other agri-food sectors. For the empirical component, a structured survey was distributed to insect industry stakeholders across the value chain. The results of the pilot survey indicate that digitalization within insect production is unevenly distributed across operational processes. Respondents identified insect counting and biomass monitoring, feeding optimisation, and process monitoring as key operational challenges where digital solutions could significantly improve efficiency and scalability. At the same time, environmental monitoring and climate control systems were reported as comparatively more digitalized, often using sensor-based monitoring and partial automation. The findings suggest that current digital adoption in insect farming is largely technology-driven and process-specific rather than strategically integrated across production systems. Based on the empirical results, the study proposes a digital maturity assessment approach for insect farming that adapts digitalization metrics used in other agricultural and industrial sectors to the specific characteristics of insect production. The proposed framework provides a methodological basis for future empirical studies and supports policymakers, researchers, and industry stakeholders in identifying priority areas where digital technologies and AI applications could realistically contribute to improved operational efficiency and economic viability in insect farming.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Pilot-scale drying of black soldier fly adults: a preliminary study for compliance to veterinary feed requirements

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End-life black soldier fly adults are an important side-stream of the black soldier fly rearing process. Valorisation of this product allows higher production sustainability and economic viability for the producers. The current EU legislation allows to feed chicken with live insects belonging to the *Hermetia illucens* species (Reg. EC 767/2009). On the other hand, when killed, insects need to be opportunely processed (Reg. EU 2017/893). This study aims to evaluate different drying processes for obtaining microbiologically safe black soldier fly adults. A pilot-scale drier consisting in 9 layers was used. Three drying operations consisting in (A) 70 to 80 °C for 4.5 h, (B) 50 to 60 °C for 24 h, (C) 40 to 50 °C for 24 h and 90 °C for 1 h, were conducted. Samples were collected every 30 min during the first 4.5 h of drying and at the end of each drying. Drying kinetics, microbial and chemical analysis were considered. Results showed that the moisture of fresh flies (50.78 ± 0.90% DM) decreased fast during the first hours of drying when 70-80 °C were applied (sample A), reaching 93.38 ± 2.26% DM after 4 hours. On the other hand, when the samples were dried at lower temperature (B), the moisture decreased slowly during the first hours (69.73 ± 0.57% DM after 4 h), and faster in the following 30 min (91.20 ± 0.48% DM). Longer treatments at 50-60 °C only showed a limited effect on the total moisture content. Application of two-steps drying (sample C) did not significantly improve the dry matter of the final samples (92.02 ± 0.36% DM) but resulted in samples with different microbial counts with aerobic mesophilic bacteria, Enterobacteriaceae and yeasts and moulds showing reduction of 1.8 log, 3.0 log, and 4.3 log, respectively. Limited microbial reductions were instead observed when A (aerobic mesophilic bacteria: –1.4 log, Enterobacteriaceae: –2.6 log; yeasts and moulds: –3.3 log) and B (aerobic mesophilic bacteria: –1.3 log, Enterobacteriaceae: –2.3 log; yeasts and moulds: –3.9 log) were applied. Application of B and C also resulted in insects with higher protein content, amounting for approx. 40% DM, against the 25% of fresh insects and the 31% of treatment A insects. No significant differences were detected on the amino-acid composition. In conclusion, a drying process for 4 h at 80 °C or for 24 h at lower temperatures (40–50 °C) followed by 1 h at 90 °C was advised for obtaining dry black soldier fly adults able to be stored for long time at room temperature and complaining the safety requirements for feed use.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Microwave-assisted extraction of insect proteins: comparative analysis of alkaline and saline conditions

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Insect-derived proteins are sustainable alternatives to animal proteins but are conventionally isolated by energy-intensive alkaline extraction with isoelectric precipitation (AEIP), while microwave-assisted and saline extraction offer intensified and milder alternatives, respectively. This study compared alkaline MAE and saline MAE in terms of protein yield, molecular weight distribution and emulsifying capacity. Alkaline MAE (0.25 M NaOH) was applied to grasshopper (GH) (*Locusta migratoria*) defatted meal during 7 minutes at absorbed energy densities (AED) from 33 to 129 J ml^{-1} and compared with AEIP (1 h) (Laroche *et al.*, 2022). Under alkaline conditions, MAE improved protein yield (from 30 to 51%), while reducing drastically reducing extracting time. SDS-PAGE showed progressively more intense protein bands from 10–180 kDa with increasing AED, consistent with enhanced solubilization of proteins that are not efficiently released under conventional AEIP. GH extracts exhibited consistently high emulsifying activity (EA ≈60%) across AED values, indicating that, microwave exposure did not compromise the hydrophobic–hydrophilic balance required for interfacial functionality. Saline MAE (0.15 M NaCl) was evaluated under comparable conditions and, then applied to GH, black soldier fly (BSF) (*Hermetia illucens*) and lesser mealworm (LMW) (*Alphitobius diaperinus*) at AED 87, 98 and 178 J ml^{-1}. Protein yields saline conditions, remained around 20%, markedly lower than alkaline MAE and consistent with limited protein solubilization at near-neutral ionic conditions (Aniyam *et al.*, 2024). SDS-PAGE revealed the appearance of higher-molecular-weight proteins (up to approx. 130 kDa) as AED increased, indicating improved extraction of protein fractions that remain embedded in the solid matrix at low energy inputs. This pattern, observed in GH, BSF and LMW, suggests that microwave-driven intensification enhances mass transfer even under moderate ionic conditions. Nonetheless, additional mechanisms, such as microwave-induced structural disruption or partial unfolding, cannot be excluded. Functionally, saline MAE extracts showed EA increasing from ≈10% to ≈40% with higher AED, reflecting the broader range of proteins mobilized during intensified extraction. Overall, alkaline MAE enhanced protein yield and functionality while reducing extraction time, whereas saline extraction highlighted complementary ionic and energy-density effects. These results support microwave-based technologies and ongoing comparisons with other intensification approaches, such as high-pressure-assisted extraction. References: Anyiam, P.N., Nwuke, C.P., Uhuo, E.N., Ajah, O., Uche, C.P., Dike, O.D. and Onyemuchara, T.C., 2024. Influence of pH and salt conditions on extraction efficiency and functional properties of *Macrotermes nigeriensis* protein concentrate for food applications. Discover Food 4: 100. Laroche, M., Perreault, V., Marciniak, A., Mikhaylin, S. and Doyen, A., 2022. Eco-Efficiency of Mealworm (*Tenebrio molitor*) Protein Extracts. ACS Food Science & Technology 2: 1077-1085.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Evaluating the impact of Grow Detect AI, an AI-driven monitoring tool on BSF production efficiency

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Large-scale insect production faces challenges such as process variability, high labor demand, and inefficient monitoring. While the sector is gradually adopting digital solutions, the use of AI-driven monitoring in black soldier fly (BSF) production remains limited. Tools such as Grow Detect AI could improve production efficiency and enhance process stability. Grow Detect AI is a modular AI-based monitoring system for black soldier fly (BSF) production that uses image analysis to count larvae, estimate larval size at different developmental stages, and assess pupal hatching patterns from representative image samples. The system operates with standard smartphone images and does not require specialized hardware when recommended image-capture protocols are followed. It can analyse images containing up to 500 larvae with detection accuracy exceeding 95%, even in the presence of small amounts of frass. However, accurate size measurements are currently limited to larvae with body lengths greater than 1 mm. This study evaluated the impacts of Grow Detect AI use for 5-day-old larvae (5 DOL) counting and size estimation on a pilot BSF farm, in a trial conducted over three production cycles. The measured KPIs included larvae estimation accuracy, labour hours for monitoring, feed conversion ratio (FCR), growth uniformity (coefficient of variation, CV), and batch-to-batch production variability. Operator feedback was collected via structured interviews to assess the usability, adoption barriers, and perceived value. The use of Grow Detect AI reduced 5 DOL larvae counting error to 3.28 ± 0.71% from 19.11 ± 0.83% in manual counting (92% counting accuracy improvement). The labour hours required for larvae counting decreases from 203 ± 6.32 s to 91.1 ± 3.47 s to count 500 larvae (45% improvement), feed application was improved through the homogenous size larvae use, resulting in a 15% improvement in FCR from 1.38 ± 0.02 to 1.20 ± 0.02. Growth uniformity improved, with the CV of adult larval weight, decreasing from 17% to 6%, and batch-to-batch production variability (total yield) decreased by 27% (variability decreased from 10.4% to 7.5%). Operators reported higher confidence in production planning and decision-making processes. Adoption barriers were adequate personnel training requirement to support the transition from manual counting procedures to a software-based workflow. Grow Detect AI improves the efficiency and productivity of Black Soldier Fly (BSF) production. Improvements in key KPIs indicate strong potential for broader adoption. These results highlight digital tools as important enablers of scalable and sustainable insect farming.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Automated image-based assessment of black soldier fly larval quality at batch level

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The larvae of the black soldier fly (*Hermetia illucens*) are increasingly recognised as a sustainable biological resource for protein and lipid production. With the scale-up of insect-based bioconversion, there is a need for robust, objective assessment of larval quality in terms of traits directly indicative of harvestable biomass (size), metabolic vitality (activity) and physiological health (colour), which are critical for predicting yield, optimising feeding regimes and ensuring suitability for downstream processing. This work presents a proof-of-concept, fully automated image- and video-based framework for low-cost, scalable batch-level quality monitoring. Low-cost RGB images and videos were acquired using a consumer-grade smartphone camera positioned above random samples of larvae drawn directly from the production process and processed with Canny edge detection and a watershed algorithm for larval segmentation, achieving about 99% agreement with manually annotated ground truth on a small, preliminary dataset without the need for high-end GPU hardware. From the segmented larvae, morphometric, colour and motion features are extracted and aggregated into interpretable indicators. For size, non-parametric, quartile-based thresholds define area-based classes (e.g. <3.3, 3.3–7.2, >7.2 mm^2). Colour scores are computed as CIEDE2000 distances to a reference colour representing visually healthy, well-fed larvae, and activity is quantified via a normalised motion history image score. The framework was evaluated on data from a laboratory imaging setup designed to be transferable to industrial practice and from pilot-scale industrial conditions during rearing and pre-harvest inspection. It performed well under standardised illumination, while larval clumping at high densities and variable ambient lighting were identified as key challenges, particularly for colour-based indicators. The resulting operator-independent indicators provide early warning signals that can support data-driven decision-making in insect factories and inform potential corrective actions such as adjusting climate or feed regimes. Systematic validation under a broader range of process conditions and substrates is still pending, and ongoing work focuses on extending the labelled data basis and refining the thresholds, including for substrate-specific colour variations.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

A low-cost larvae counter for black soldier fly systems

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Accurate dosing of black soldier fly larvae is a critical control point in both grow-out and reproduction systems. The number of larvae used as stocking material in relation to the amount of substrate fed directly determines growth performance, survival rates, and the predictability of work processes. Despite its importance, reliable quantification of young larvae remains difficult in practice. Larvae are small, highly mobile, and typically mixed with heterogeneous feed residues and frass. Manual counting of samples is therefore time-consuming and unpopular with workers. As a result, this step is often bypassed and operators rely on rough weight-based estimates, introducing substantial uncertainty into process control. This contribution presents the development of a larvae counting system designed to automate the quantification of representative samples of stocking larvae while remaining simple to operate under field conditions. The system combines a mechanical pre-processing step based on passive sieving with an automated counting unit using controlled funneling and machine-vision-based detection implemented on a compact camera and single-board computer platform within a closed, illuminated counting chamber. Experimental validation was conducted by comparing automated counts with manual reference counts across a total of ten trials covering different operating conditions and larval size ranges. Tested larvae covered an average individual weight range of 0.85–5.3 mg. Initial experiments resulted in substantial overcounting (up to 147%), caused by the misclassification of non-larval particles such as frass. Following adjustment of detection thresholds to supress noise, repeated trials (n = 3) with sample sizes ranging from 613 to 762 larvae (0.84–0.87 mg per larva) demonstrated a counting accuracy of 96–100%. The observed counting duration ranged between 21 and 25 min/sample, with most larvae being counted within the first 10 min. Pre-processing required approx. 20–25 s/g mixed sample. Ongoing development focuses on improving the feeding mechanism to increase larval flow and reduce total counting time. Application areas include routine sampling in reproduction and grow-out operations, where improved quantification of stocking larvae enables more accurate dosing, higher process reliability, and contributes to the professionalisation of the black soldier fly technology.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Integrating bioacoustics into multi-sensor monitoring systems for precision insect farming

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Monitoring larval behaviour and health in black soldier fly (*Hermetia illucens*) production systems is challenging because larvae develop within opaque substrates and at high stocking densities, limiting direct observation. While visual monitoring systems can measure the larval size, number, and growth, they provide limited insight into behavioural activity. This study proposes a hypothesis-driven monitoring framework to investigate whether acoustic emissions produced by larval movement and feeding can serve as indirect indicators of stress, overcrowding, or suboptimal rearing environments. Larval movement and feeding produce low-amplitude vibrations through cuticle friction and particle movement within the substrate. Similar bioacoustic approaches have been used to study behavioural dynamics in insects such as bees, termites, and crickets, indicating that acoustic activity may provide measurable proxies for colony behaviour. We present a methodological framework that integrates passive acoustic sensing into a multi-sensor monitoring system for insect farming. Acoustic sensors pick up sounds when the larvae move around in the substrate. These signals are synchronised with environmental measurements and process metadata. Signal analysis extracts activity-related acoustic features such as signal energy, dominant frequency bands, and temporal activity rates. These acoustic descriptors are combined with environmental variables through data fusion to support behavioural inference. Conceptual signal analyses demonstrate that acoustic feature extraction can reduce monitoring data volumes by roughly 70–80% compared to image based analysis. Activity-related acoustic indicators are expected to capture behavioural changes associated with feeding intensity, larval density, and environmental stress. For example, a drop in acoustic activity may indicate decreased feeding due to substrate degradation, while an increased activity patterns may signal overcrowding or agitation under suboptimal environmental conditions. These behavioural indicators could support operational decisions such as adjusting feed moisture, replacing degraded substrates, or modifying stocking density before measurable declines in larval growth occur. However, acoustic monitoring provides indirect behavioural proxies rather than direct biomass measurements. The proposed framework positions bioacoustic sensing as a complementary modality for precision insect farming. Continuous, non-invasive monitoring of larval activity may improve early detection of suboptimal rearing conditions and support data-driven operational decisions in BSF production, providing a basis for future experimental validation.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Prophylactic efficacy of bacteriophages against *Salmonella* spp. in black soldier fly larvae rearing systems

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Various organic waste streams can be utilised as feed for black soldier fly larvae (BSFL) but potentially harbour pathogenic *Salmonella*. Bacteriophages, which are natural enemies of bacteria, have arisen as a potential biocontrol strategy for *Salmonella* contaminations in the meat processing industry, e.g., sprayed on poultry carcasses. Yet, their application and efficacy in insect-rearing environments is largely unexplored. This study aimed to provide a proof-of-concept for using bacteriophages as a prophylactic administration strategy for targeted mitigation of *Salmonella* in BSFL rearing. In a challenge experiment, the following four conditions were tested, with each replicated n = 3 times: (i) 200 g of standard BSFL diet (1:1 chicken feed to water); (ii) the same amount of diet to which 500 7-day-old larvae were added; (iii) feed-only inoculated with a mixture of two pathogenic *Salmonella* (7 log CFU/g, 1:1 *S. typhimurium* and *S. infantis*); and (iv) diet with 500 larvae and the pathogen mixture. These four conditions were duplicated, with one set treated with a commercially available bacteriophage cocktail, Phageguard S. (9 log PFU/g), and the other set being untreated. The presence of both *Salmonella* and bacteriophages within the substrates and the tissues of surface-sterilized BSFL were destructively sampled on days 0, 2 and 6 of the experiment. Results indicate that treatment with the bacteriophage cocktail led to a substantial reduction in *Salmonella* counts (3 log CFU/g) within as little as 1h post-treatment. For treatments without larvae present in the substrate, pathogen counts continued to decline over the experimental period, reaching the detection threshold (<3.0 log CFU/g). However, in the presence of larvae, the counts recovered by day 6 of the experiment (6.4 ± 0.3 log CFU/g). The decline of phage numbers over time in the substrate, on the other hand, was slower in the presence of larvae. The results also confirmed that functional *Salmonella* phages could be retrieved from the larvae after day 2 and 6 of the experiment. Interestingly, these findings suggest that larval presence may contribute to phage persistence over time, but counter-intuitively this is not associated with a more efficient reduction of *Salmonella*. As such, this proof-of-concept study showcases the potential of phage therapy as a control strategy to reduce the concentration of unwanted microbes in insect production systems, but also highlights several research questions that need to be addressed (e.g. how larvae impact both phage infectivity and pathogen dynamics).

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Black soldier fly adults spatial distribution by age, weight, and love-cage environmental conditions

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Industrial breeding cages for black soldier flies (BSF) are designed to increase and ensure stable, high-quality egg production. To achieve this goal, several factors must be considered (environment, population dynamics, physiological fly parameters, and artificial habitat structure), but they are often analysed separately rather than together. This research aims to evaluate how the spatial distribution of flies in the love-cage was affected by environment (temperature (T) and humidity (RH)) and physiological fly parameters (age, weight, and sex). Inside a 24 m^3 (6 × 4 × 1 L × W × H m) climatic chamber with constant T (27 ± 1 °C), RH (65 ± 5%), a 1.5 × 2 m resting area was used to assess fly spatial behaviour. For data collection, the area was divided into three zones (0.73 × 1.5 m each) at heights of 36,106, and 176 cm from the ground. To verify the behavioural differences during the day, sampling was done twice: at 9.30 am and at 2.30 pm. The experiment was repeated for two consecutive weeks during the spring months of 2024. During sampling from each zone of the resting area, T and RH of were measured, and 10 specimens were taken, individually sexed, weighted, and estimated their age by quantifying the fat reserves present in the abdominal 'window'. Window fullness is an indicator of age: value close to 1 indicate young flies, while values closer to 0 indicate older ones. To understand the distribution of individuals on the resting surface, all parameters of adult BSF (sex, weight and age) were correlated with T and RH of each zone. Considering the entire resting area, 60% of the individuals sampled were female. Regardless of the sampling zone, the weight of adults was strongly and positively correlated with the age. Indeed, females were heavier (41.93 ± 18.79 mg) and with more fat reserves (so younger) than males (36.95 ± 13.45 mg) ($p < 0.005$). Negligible correlations were found among the weight and age of adults and the environmental parameters (T and RH). Nevertheless, regression models revealed a significant increment in the fat resource with increased RH was found in BSF females. In conclusion, in industrial system young BSF female use resting areas more frequently than males' ones, and RH influences their spatial distribution. Therefore, it can be inferred that the female specimen requires energy derived from fat reserves and a proper RH environment for oocyte maturation. This could be a good starting point for future research.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Economic performances of small-scale insect dry larvae production: a comparison between control and waste-based diets

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The high market price of insect-based proteins remains a key barrier to large-scale adoption in animal feed, despite their recognised environmental and nutritional potential. In this context, improving the economic sustainability of insect farming is crucial, especially for small- and medium-scale production systems typical of Europe. This study evaluates the economic performance of black soldier fly (*Hermetia illucens*) meal production under two feeding strategies: a diet based on commercially available feed -Gainesville diet- (case study 1), and a waste-based diet based on selected agri-food by-products (case study 2). Data were collected from a pilot-scale insect rearing and low-tech processing facility and analysed through full cost accounting, separating fixed costs (infrastructure, machinery, financial charges) from variable costs (labour, feed inputs, energy use, water, consumables, process control and quality assurance). Costs were expressed as € per kg of dried larvae at pilot scale. Results show that labour represents the dominant cost component in both scenarios, accounting for more than 60% of total production costs and increasing to over 70% under the waste-based diet. In the conventional scenario, feed inputs represented around 15% of total costs; when by-products were used as feed, their contribution became marginal (approx. 1–2%). Fixed costs -including infrastructure, machinery, and financial charges- remained relatively stable across scenarios, indicating limited short-term flexibility in small-scale systems. Overall, the waste-based diet substantially reduced variable costs and improved cost efficiency. However, the high incidence of labour and capital-related expenses suggests that further competitiveness will depend on increased automation and scale expansion. In addition, heterogeneous by-products may require extra management to maintain nutritional consistency and process stability. Integrating waste recovery into insect diets can enhance economic sustainability and support local circular feed supply chains; nevertheless, at pilot and small-scale, production costs remain higher than those of conventional protein sources. These systems should therefore be regarded as a promising pathway towards more resilient and environmentally sustainable feed supply chains. Overall, the waste-based scenario (2) outperforms the conventional feeding strategy in terms of cost efficiency, but its competitiveness ultimately depends on structural changes in production systems rather than feed substitution alone.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Defatting of *Acheta domesticus* by supercritical fluid technology

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Despite the high protein content of insects such as *Acheta domesticus*, defatting processes can be performed to obtain products with an even higher protein concentration because of their high lipid content. This process generates a substantial volume of lipid co-product, which is increasingly attracting interest owing to its valuable nutritional composition, particularly its high degree of unsaturation, such as *A. domesticus*. Traditionally, defatting is performed using organic solvent extraction or mechanical pressing; however, alternative methods such as supercritical fluid extraction (SFE) are emerging as cleaner and more selective options. This study focused on developing a sustainable SFE method for the defatting of *A. domesticus* and on the characterisation of the two main products obtained: fat and defatted powder. A traditional method of defatting using hexane was compared to SFE. Different combinations of pressure (350 or 450 bar) and temperature (40 or 60 °C) at fixed CO_2 flow of 80 g/min were tested. Kinetics of defatting were obtained under the 4 tested conditions during 60 min of extraction. Proximate composition of the defatted powder and the fatty acid profile and minor lipid compounds of the lipid fraction was performed. The condition of 450 bar-60 °C was the most efficient method of defatting, producing the highest oil yield (22%) at the shortest time of extraction (40 min). After these conditions, the derived defatted powder consisted of 74% protein, 13% carbohydrates, 5% lipids and 5% ashes. The notable concentration of proteins due to the SFE defatting process was evidenced, since the starting material (cricket powder) consisted of 63% protein, 19% lipids, 7% carbohydrates and 4% ashes. With respect to the lipid obtained co-product, this showed the typical fatty acid profile for *A. domesticus*, with a similar content of saturated (35%) and unsaturated fatty acids (43%), with linoleic acid (40%), palmitic (26%), oleic (21%) and stearic (9%) acids as the most abundant. Additionally, minor compounds such as cholesterol, desmosterol, and stigmasterol were detected, offering valuable insights into the lipid composition. Therefore, this study demonstrates that SFE can be a sustainable and efficient alternative for defatting *A. domesticus* without compromising the conventional nutritional composition of the derived products.
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INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Supercritical CO_2 for sequential defatting and production of bioactive extracts from *Acheta domesticus*

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Acheta domesticus (AD) is an edible insect approved for food and feed rich in proteins, lipids and micronutrients. Beyond its nutritional value, AD has also been reported as a source of minor compounds associated with bioactive effects, such as antioxidant, anti-inflammatory and hypolipidemic properties. The production of insect extracts with high concentration of bioactive compounds and enhanced biological activity often requires a prior defatting step. In addition, the appropriate selection of extraction techniques is crucial to maximising extract bioactivity. In this context, supercritical CO_2 ($SCCO_2$) is a sustainable alternative to conventional defatting methods based on the use of toxic and polluting solvents. Additionally, $SCCO_2$ allows the extraction of bioactive compounds from the defatted material. The aim of this study was to evaluate the potential of $SCCO_2$ for sequential defatting and production of bioactive extracts from AD. Extractions were carried out sequentially in two stages. First, neat $SCCO_2$ (350 bar, 40 °C, 80 g/min, 60 min) was applied for the defatting of AD powder. Subsequently, a second extraction was performed on the defatted material using two different $SCCO_2$ flow rates (60 and 100 g/min) and EtOH concentrations (10% and 20%) as co-solvent. Total phenolic content (TPC. Folin-Ciocalteu method, as g of gallic acid equivalent (GAE)/100 g extract) and the antioxidant (DPPH and ABTS assays) and anti-inflammatory (lipoxygenase -LOX- inhibition) activity of the extracts were determined and expressed as IC_{50} values. The defatting step resulted in a lipid removal of 84.7%. The second extraction step for obtaining bioactive extracts showed that the extraction yield increased with both CO_2 flow rate and EtOH concentration, reaching a maximum of 4% at 100 g/min and 20% EtOH. A similar trend was observed for TPC, antioxidant and anti-inflammatory activities. Thus, the highest TPC value (15.8 mg GAE/g extract) was obtained at 100 g/min and 20% EtOH, together with the lowest IC_{50} values for ABTS (405.5 μg/ml), DPPH (690.3 μg/ml) and LOX inhibition (433.1 μg/ml). These results highlight the potential of $SCCO_2$ as a sustainable and efficient method for sequential defatting and production of bioactive extracts from AD that could be used for the development of insect-based ingredients for health. Acknowledgement: Funded by the European Union under grant agreement No 101162517. Call: HORIZON-EIC-2023-PATHFINDERCHALLENGES-01.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Mislabelled insect-based products: use of Near Infrared Spectroscopy techniques to ensure quality control

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Over the past few years, a wide range of insect-based products has emerged on the international and European markets, promoted for their nutritional benefits and low environmental impact. Although these products represent a major economic opportunity, they are currently subject to very limited quality control. Consequently, a recent study revealed that, among one hundred insect-based products analysed using genomic methods, the majority exhibited labelling inaccuracies. However, genomic methods require the use of expensive products and involve time-consuming sample preparation. To develop rapid and non-invasive approaches for assessing the quality of insect-based products available on the market, two Near Infrared Spectroscopy (NIRS) techniques were applied on commercially feedstuffs containing one or more insect species. First, a Near Infrared Microscope (NIRM) was used to authenticate insect species present in the samples. Second, NIRS was employed to evaluate protein, lipid and moisture contents of feedstuffs. The same samples were also analysed using real-time PCR, metabarcoding and conventional wet chemistry analyses to accurately assess the performance of NIRM and NIRS, respectively. The results obtained using NIRM and NIRS enabled the detection, authentication and compositional evaluation of the analysed insect-based products and were consistent with those obtained by real-time PCR and wet chemistry analyses. By comparing the different analytical results with the declared compositions on product labels, several labelling discrepancies were identified. NIRM analyses revealed errors related to the insect species, including the presence of species different from those declared or the complete absence of certain species despite being listed on the labels. Furthermore, NIRS analyses highlighted substantial discrepancies in protein content for several samples, with measured values around 9% compared to the 55% indicated on the labels. Overall, these findings underscore the importance of establishing robust quality control analysis for insect-based products, not only to prevent fraud but also to support the sustainable development of this emerging sector.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Supercritical fluid extraction of *Tenebrio molitor* larvae fed on rice crop byproducts rearing substrates

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The Sustainable Development Goal 2 aims to achieve food security, improve nutrition and develop sustainable and resilient food production systems. Edible insects (EIs) were recognised as alternative sources of nutrition with lower carbon and water footprints than traditional livestock. Their consumption could even be more beneficial to sustainability if they are reared on agricultural byproducts. Among EIs, *Tenebrio molitor* larvae (TML) are abundant in nutrients like proteins, fatty acids, minerals, and vitamin C. EFSA approved TML for use as a food and feed in European countries. Nevertheless, consumers' acceptance remains low. Studies suggest it will increase if EIs are incorporated into food, such as bakery products. This study aims to obtain a rich protein flour from TML grown on rice crop by-products for use in baked products, including its polyunsaturated fatty acid-rich fraction. TML were grown on crop rice byproduct rearing substrates containing 10.5% white rice byproducts. TML were freeze-dried and finely minced. To separate the protein from the oil fraction, CO_2 supercritical fluid (SC-CO_2) extraction was performed. This extraction technique efficiently produced food-grade solvent-free extracts. In this study, two different methods were applied. In method I, the chosen process parameters were 400 bar and 65 °C. A 50-ml extraction vessel was filled with minced TML flour, and SC-CO_2 was provided. A first extraction phase was conducted in batch mode for 50 minutes, followed by continuous SC-CO_2 flow for 40 minutes to collect the extracted oil. In method II, the parameters chosen were 300 bar, 65 °C in a 500-ml extraction vessel. Flours with two degrees of defatting were obtained: 90% defatted (fl10) and 45% defatted (fl65). Based on dry matter, results indicate that they contained 59% and 47% crude protein, respectively. The results obtained are within the range reported in the literature (47 – 60%) for SC-CO_2 extraction. According to the data, method I allows the production of a flour with a higher protein content. Nevertheless, fl65 could also offer advantages for the preparation of baked products from a technological standpoint. Thus, the oil and protein fractions were characterised and used to prepare preliminary baked products. Research supported by Fondazione Cariplo and Compagnia San Paolo (newRIFF and TOWINBAKE projects).

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Support for the implementation and development of NIRS technology for the insect sector

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Near-infrared spectroscopy (NIRS) is an analytical technique that relies on infrared light absorption to generate an electromagnetic spectrum, producing a unique "spectral fingerprint" associated with chemical composition. By establishing correlations between spectral data and chemical properties, NIRS technology enables predictive modelling of organic materials. After calibration, this approach allows accurate estimation of key nutritional components such as proteins, dry matter, lipids, and sugars in substrates, frass, and insect larvae at different processing stages. This technology offers significant advantages for industrial insect production. On the one hand, in R&D applications involving highly variable samples of substrates, larvae, and frass, we obtained robust equations that enable real-time substrate control, as well as high-throughput analysis of frass and larvae within short time frames. For example, for protein content in substrate (ranging from 8 to 30%), using 130 samples with chemical reference analyses and four years of R&D improvements (now 325 samples), we achieved very satisfactory results: n = 325; slope = 1.01; SEC = 1.55; R^2 = 0.91; RPD = 2.44. The chemical uncertainty is 1.2, and the maximum target for NIRS is an SEC of 1.4. On the other hand, in industrial applications—where variability is lower but real-time measurements are required on production lines with high throughput and the ability to scan a large share of the output—we also achieved strong performance. For example, for fat content in live larvae (ranging from 7% to 15%), using 35 samples and 10 months of development, the results were satisfactory: n = 35; slope = 1.06; SEC = 0.8; R^2 = 0.96; RPD = 3.85. The chemical uncertainty is 0.8, and the maximum target for NIRS is an SEC of 1.0. In routine use, ongoing improvements have increased the calibration set to 110 samples. NIRS enables reduced costs compared with conventional methods. Its implementation supports precise monitoring of production cycles and improves process control, ultimately leading to better product characterisation and enhanced operational efficiency. Supported by Le Gouessant, NIRS can be deployed at both laboratory scale and online production environments, contributing to the optimisation of insect-based bioprocesses.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Oil extraction from black soldier fly larvae: impact of dehydration method and extraction process on yield and recovery

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Black soldier fly (BSF, *Hermetia illucens*) larvae represent a promising lipid source for feed and non-feed biorefinery applications. This study evaluated the influence of dehydration methods and extraction strategies on oil recovery from BSF larvae, comparing aqueous extraction with the green solvent 2-methyltetrahydrofuran (2-MeTHF) as an alternative to conventional hexane. Larvae were dehydrated by microwave (MW; 100 °C, 20 min), oven (OV; 65 °C, 24 h), or freeze-drying (FD; 48–72 h). The resulting samples showed dry matter contents between 95.9% (OV and FD) and 96.9% (MW), while crude fat contents ranged from 32.6% (FD) to 34.3% (MW). Oil extraction results showed a clear difference between methods. Extraction with 2-MeTHF achieved about 35% oil yield corresponding to nearly complete lipid recovery (≈100%), regardless of dehydration method. In contrast, aqueous extraction showed strong dependence on pre-treatment. FD samples formed stable emulsions preventing phase separation and oil recovery, while OV- and MW-dried larvae allowed partial oil recovery with significantly lower performance (6% yield and 17% lipid recovery for OV; 16% yield and 45% lipid recovery for MW). These results demonstrate that dehydration method critically affects aqueous extraction efficiency due to emulsion formation, whereas 2-MeTHF enables consistently high lipid recovery. The performance obtained with 2-MeTHF was comparable to oil yields reported for hexane extraction in the literature, while offering improved safety and environmental performance. Further work will focus on improving aqueous extraction by reducing emulsion formation to enhance oil recovery and enable its integration as a complementary low-cost strategy in BSF biorefineries.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Valorisation of mealworm exuviae as a sustainable source of chitosan

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The growing need for sustainable food systems has intensified interest in insect farming as an efficient source of protein for human food and animal feed. In addition to their nutritional value, insects generate by-products that offer further opportunities for resource valorisation. In this context, the larval exuviae of *Tenebrio molitor*, a by-product of mealworm production, represent an underutilised biomass with high potential for conversion into value-added materials. In this study, chitosan was extracted from *T. molitor* larval exuviae using sequential demineralisation, deproteinisation, and deacetylation. The chitin yield from exuviae reached 12.86%, while the chitosan yield was 7.41% relative to the initial dry biomass, corresponding to 57.76% of the extracted chitin. The obtained chitosan exhibited a medium degree of deacetylation (72.27%) and a viscosity-average molecular weight of 612 kDa. High product purity was confirmed by low residual ash (0.33%) and amino acid content (0.14%). Morphological analysis revealed a fibrous and porous microstructure, particularly after purification. Chitosan films prepared from a 1% solution were uniform and transparent, with low thickness (0.0197 mm), high swelling capacity (335.07%), moderate water solubility (46.99%), and moisture content of 32.39%, indicating good film-forming ability and functional performance. These properties support the applicability of insect-derived chitosan in biodegradable films and other advanced applications. The results demonstrate that by-products of insect farming can be effectively integrated into circular bioeconomy models. Valorisation of *T. molitor* exuviae not only reduces waste from insect-based food and feed production but also enhances the overall sustainability of insects as a resource for feeding the world. Acknowledgements: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Project Nos. 451-03-136/2025-03/200222 and 451-03-136/2025-03/200134) and by the Croatian Science Foundation (CSF(HRZZ)) and Slovenian Research Agency (ARIS) under the Weave initiative project nos. HRZZ IP-2022-10-8305 and ARIS N4-0346 (PROGRESS) and under CSF project HRZZ-DOK-2021-02-5517.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Frass or feed? Analytical challenges in insect meal quality control

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Within the framework of the EU Green Deal and the Circular Economy Action Plan, reducing dependence on critical feed materials and limiting food waste are key objectives. In this context, insect meal has emerged as a promising alternative protein source for livestock feed. However, due to higher production cost compared to conventional proteins, its use in animal nutrition remains limited. Frass, a co-product of insect farming composed of faeces, residual substrate, dead insects and cuticular fragments, is produced in large quantities. Under economic pressure, some producers may be tempted to adulterate insect meal with frass to increase volume and reduce costs. Once mixed, frass is visually indistinguishable from insect meal, and no official analytical method currently allows its detection. This study investigates how proteomic analysis could identify and quantify frass adulteration in insect meal? The reliability of proteomics depends on efficient and representative protein extraction, yet no optimised protocol exists for frass. Developing a robust extraction procedure is therefore essential for achieving sensitive and reproducible analyses. This work focuses on optimising protein extraction from *Hermetia illucens* frass. Insects were reared on plant-based substrates and harvested at three developmental stages (6-DOL, 13-DOL and pupae). Insects and frass were collected, dried and ground. Four extraction protocols were tested, combining two urea concentrations (low/high) with or without a cold-acetone defatting step. Protein yields were then quantified. High urea concentration consistently improved extraction efficiency for both insect meal and frass. In insect meals, defatting further increased protein yields, whereas in frass it had the opposite effect, reducing extracted protein quantities. These trends were identical across developmental stages, confirming their robustness. These results show that extraction conditions are a critical source of variability in proteomic workflows. However, protein quantification alone cannot predict whether these conditions will also allow the detection of specific peptide markers distinguishing frass from insect meal. Therefore, all samples will now be analysed by UHPLC-MS/MS to verify whether the protocols maximising protein yield also optimize marker identification. This preliminary work lays the methodological basis for developing a reliable proteomic method to detect frass adulteration in insect meal.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Insect counting system on production line

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The insect industry is facing many challenges to improve its operational performance. One of the them is to measure the number of animals used per tray of production as it was mainly evaluated by a mean weight bringing to much variability and uncertainty. Moreover quality control was performed manually by operators and this step was time consuming and with a questioning reliability. Ideally the industry was looking for a system to be implemented directly on the production line without compromising the productivity rate. The insect counting system was designed to perform an exhaustive (95%) count of insect larvae of few millimetres in farming, directly on a production conveyor at 25m/min without separation from their feeding substrate and the full population of each tray. The development relied on a thorough selection of waves lengths (infrared and visible lighting), image acquisition with a specific camera (resolution and speed) and paired with deep learning-based analysis (trained on an important dataset labelled by experts). The device enables the distinction between all type of substrate, live and dead larvae and adapts to various industrial conditions (conveyor speed, width, and substrate type). The only constraint is to implement the equipment on a part of the process delivering a thin layer of substrate to avoid larvae to be totally hidden by the substrate particles while counting. Results demonstrate a high counting accuracy (>95%) with a variation coefficient below 1% (assessed on 10 samples per 10 repetitions). These results are reaching the performance targeted by the industrial as it is above both the accuracy and consistency delivered by the former system on place. Continuous learning ensures long-term consistency, paving the way for further applications in automated insect farming. The technology is patented and is also implemented for R&D operations.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Comprehensive characterisation of adult insects to assess their potential for sustainable biorefinery processes

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A large part of current research focuses on the larval stage of insects. However, the adult stage can also be valorised. Through insect bioprocessing, insects can be separated into several fractions: protein, lipids, and chitin. This work focused on carrying out a comprehensive characterisation of adult insects: *Acheta domesticus* (AD), *Locusta migratoria* (LM) and *Hermetia illucens* (HI) to evaluate their potential in biorefinery processes. The three adult insects were characterised in triplicate as follows: protein (after subtracting the nitrogen of chitin origin), lipids, chitin, ash, and moisture content. Also, amino acids, fatty acids and minerals profile were assessed, as well as the *in vitro* digestibility of protein mimicking human digestion conditions. AD exhibited higher protein (73.5% dry weight, DW) content, which is 15% and 25% more than HI (49.4% DW) and LM (45.3% DW), respectively. Contrarily, higher lipids content was observed in LM (31.4% DW), followed by HI (20.1% DW) and AD (6.89% DW). Chitin content was similar among AD and HI, ranging between 9.3% (HI) and 10% DW (AD), with lower amount found in LM (4.4% DW). Higher essential amino acids were found in AD (19.9% DW), followed by HI (13.9% DW) and LM (9.57% DW). Among the three species, AD exhibited lower saturated fatty acids (36% DW; SFA) and higher polyunsaturated fatty acids (46.2% DW; PUFA) content, while HI has a higher content in SFA (76.4% DW) and LM showed the lowest PUFA content (11.9% DW). The *in vitro* digestibility of protein was higher in AD (63.8%), followed by HI (58.0%) and LM (53.3%). Regarding minerals, high sodium (6.13 g/kg DW), calcium (1.45 g/kg DW) and potassium (12.9 g/kg DW) levels were found in AD, while HI was an important source of potassium (10.6 g/kg DW) and magnesium (4.03 g/kg DW). It is not uncommon to observe a wide range of protein values in insects reported in literature. In this work, nitrogen of protein and chitin origin was quantified, permitting not overestimate the protein content. Also, *in vitro* digestibility results highlight the promising applicability of each insect species, highlighting the importance of AD for foods formulations. These results highlight the strong potential of these insect species, particularly as versatile and sustainable feedstocks for biorefinery processes, enabling the efficient production of multiple high-value products such as proteins, lipids and chitin.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Effect of larvae inoculation weight and substrate type on development and bio-conversion rate of black soldier fly

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Productivity of black soldier fly larvae (BSFL) is strongly influenced by larval inoculation density and substrate characteristics. Low larval densities may lead to inefficient nutrient use, whereas high densities increase competition and reduce performance. This study therefore assessed the effects of substrate type, substrate depth, and larval inoculation density on BSFL growth and yield. In a completely randomised design with triplicates, 2.5 g of BSFL eggs were inoculated into six substrates to evaluate hatchability and development during the first six days. Substrates were placed in plastic basins (30 × 27 × 12 cm) at depths of 0.75, 1.50 and 3.00 inches at an average temperature of 27 °C and relative humidity of 54%. Six-day-old larvae (6DOL) were transferred into nine grower substrates, of plant- and animal-based origin, at inoculation levels of 20.8, 26.0, 31.2, 36.4 and 41.6 g. Growth, yield, and substrate bioconversion were assessed. Maize bran produced the highest larval weight among starter substrates, followed by fermented soy residue. A substrate depth of 3 inches produced comparable larval yields to other depths while generating the highest compost output. Brewery waste, poultry manure, Irish potato peels, and banana peels resulted in the greatest larval biomass. Inoculation rates above 31.2 g per 3 kg of substrate significantly reduced larval weight and overall yield. Larval growth at day 21 did not differ significantly from day 18. Irish potato waste, pineapple waste, and brewery waste recorded the highest bioconversion rates and orange peels and cow dung the lowest. Moderate larval densities (31.2 g) enhanced growth through improved aggregation and feed utilisation, while excessive densities caused competition and reduced nutrient availability. Growth peaked between days 9 and 12 due to fresh substrates with higher nutrient and moisture content and improved feeding capacity. Reduced growth after day 15 was associated with nutrient depletion and the onset of pupation. Inoculation of 2.5 eggs in maize bran starter feed, followed by inoculation of 31.2 g of 6 DOL in 3 kg of a '3 inch high' plant-animal substrate mixture, and harvesting after 12 days results in the best performance.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Impact of post-harvest treatment on the yellow mealworm (*T. molitor*) metabolite profile

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The quality of the produced edible insect is influenced by farming practices, including the choice of feeding substrate. However, post-harvest treatment has a significant impact on final product composition. In our study, three different post-harvest treatments were investigated. yellow mealworm (*T. molitor*) larvae were euthanised by (i) boiling in water (100 °C, 5 min), (ii) freezing (–20 °C, 24 h) and (iii) microwave treatment (800 W, 1.5 min). Subsequently, each variant was dried to constant weight using microwave drying (800 W), conventional hot-air drying (60 °C) and freeze-drying (–55 °C, vacuum). All samples, including lyophilised bath (water) obtained by boiling larvae, were examined by untargeted fingerprinting using ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometry (U-HPLC-HRMS/MS). Advanced statistical methods, including multivariate statistical analyses (PCA, PLS-DA), were used to evaluate the data. The results showed that insect samples euthanised by freezing and microwave contained a higher abundance of polar metabolites than those killed by boiling. The reason might be the transfer of some water-soluble compounds, such as amino acids or short peptides, into the boiling bath. Analysis of the amino acid profile confirmed the differences among the three post-harvest treatments of yellow mealworm (*T. molitor*) larvae. The results of the study may contribute to the optimisation of post-harvest treatment of edible insects for food purposes.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Intelligent feeding method for automated insect rearing

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Edible insects such as *Tenebrio molitor*, *Hermetia illucens* and *Acheta domesticus* can provide protein with relatively low environmental impacts (European Parliament, 2024; van Huis, 2013). Global protein demand for food and feed is projected to rise by about 70% by 2050 (FAO, 2017), and the insect protein market is expected to grow strongly (SkyQuest, 2026). In this context, the InFueSekt sub-project develops a *T. molitor* reproduction module with an integrated, barrier-based feeding concept. In standard crate-based reproduction, beetles and feed, as well as eggs and frass accumulate in a single layer, causing high manual effort for separation, egg losses and limited hygiene control. InFueSekt replaces this with a four-layer barrier design: (i) a substrate layer, (ii) a beetle layer with minimal substrate, (iii) an egg collection layer and (iv) a frass collection layer. Dry feed is sieved from above onto the beetle layer as particles with defined size, large enough not to pass through egg and frass sieves, but small and rounded enough not to injure beetles and to ensure access. Sieves between the layers allow eggs and frass to fall into separate zones while retaining beetles and feed above. In replicated trials, the barrier-based module is benchmarked against a conventional crate baseline. Beetle survival, oviposition, egg recovery, egg damage, hatch rate and early larval performance are quantified, and offspring quality is characterised via selected nutrient and microbiological parameters. Handling time and suitability for decentralised on-farm use are recorded alongside technical indicators. As eggs have the highest value density in the mealworm chain, the module targets improved egg yield, recovery and hygiene as a basis for scalable, decentralised breeding of *T. molitor*. The project is funded by the German Federal Ministry of Food and Agriculture (BMEL) within the innovation programme “Nachhaltigere menschliche Ernährung”. References: (1) European Parliament, 2024. Alternative protein sources for food and feed. Available at https://www.europarl.europa.eu/RegData/etudes/STUD/2024/757806/EPRS_STU(2024)757806_EN.pdf. FAO, 2017. The future of food and agriculture. DFAO, Rome. SkyQuest, 2026. Insect protein market report. Available at https://www.skyquestt.com/report/insect-protein-market. van Huis, A., 2013. Potential of insects as food and feed. Annual Reviews of Entomology 58: 563-583.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Calcium fortification of *Tenebrio molitor* larvae: powder mixing versus spray application

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Low calcium (Ca) content and inverted Ca:P ratios are major nutritional limitations of *Tenebrio molitor* larvae when used as feeder insects or as ingredients in compound feeds, since they may contribute to mineral imbalance in animal diets. This study evaluated two Ca fortification strategies by comparing wheat-bran diets nominally supplemented with calcium carbonate ($CaCO_3$) at 4, 8 and 12% (w/w): dry powder mixing (POW) and spraying an aqueous $CaCO_3$ suspension onto the substrate followed by drying (SPR). Larvae were fed the experimental diets for 48 h (ad libitum), a short-term feeding interval selected based on previous biofortification studies to enable comparison with the literature, and then fasted for 48 h. Calcium concentrations in diets and larvae were quantified by flame atomic absorption spectrophotometry. Dietary Ca increased strongly with $CaCO_3$ inclusion in both series, with all fortified diets higher than the control ($p < 0.001$). At 4 and 8% $CaCO_3$, SPR diets contained more Ca than the corresponding POW diets, whereas no difference was observed at 12%. Larval Ca also increased significantly with inclusion level ($p < 0.001$): relative to control larvae, POW diets produced approximately 1.7 – 2.7-fold enrichment and SPR diets produced approximately 2.5 – 3.4-fold enrichment. Two-way ANOVA confirmed significant effects of fortification strategy and inclusion level, with SPR consistently yielding higher larval Ca than POW at the same $CaCO_3$ level. These results demonstrate pronounced mineral plasticity in *T. molitor* and identify spray-based $CaCO_3$ application as an effective short-term approach to produce calcium-fortified larvae with improved mineral balance, supporting their use in animal nutrition.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Optimisation and modelling of protein extraction from *Tenebrio molitor* larvae

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This study examines the kinetics and efficiency of protein extraction from *Tenebrio molitor* larvae using an integrated processing sequence comprising blanching, drying, hydraulic pressing and grinding, followed by aqueous extraction under controlled operating conditions. Extractions were conducted at temperatures of 20, 40 and 60 °C and alkaline pH values of 7, 10 and 12. Kinetic analysis revealed a biphasic extraction mechanism consisting of an initial rapid washing stage, with characteristic times of ≤4 min, dominated by convective mass transfer, followed by a slower diffusion-controlled stage (characteristic times ≥30 min) governed by intraparticle transport within the larval matrix. Protein extraction yield was strongly influenced by both temperature and pH. At neutral to moderately alkaline conditions (pH 7 – 10), increasing temperature enhanced extraction efficiency due to improved protein solubility and diffusivity. In contrast, at pH 12, extraction yields declined above 40 °C, likely as a result of thermally induced protein aggregation and partial denaturation. Overall, the results define a scalable and environmentally benign extraction strategy that enables efficient protein recovery from insect biomass. This approach demonstrates strong potential for sustainable food ingredient production and the valorisation of insect-based agro-resources.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Practical case studies of black soldier fly production

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Insect-based feed systems offer significant potential to upcycle organic by-products into locally produced protein for animal nutrition. This study presents an analysis of six black soldier fly (BSF) production systems implemented across European agricultural value chains using modular and scalable factory concepts. The systems range from rearing-only units to fully integrated facilities combining breeding, rearing, and processing, operated under both manual and automated conditions. These factories are currently converting over 100 000 Tons of organic material into insect protein. A comparative framework was applied considering customer type, substrate streams, production scale, integration level, end-product form, and regulatory context. Two dominant implementation models were identified: (1) industrial-scale production of processed insect protein by agro-industrial actors, and (2) decentralized, smaller-scale systems enabling on-farm use of live larvae in livestock feeding. The results demonstrate that system configuration such as integration level, automation, and output form determine how BSF production contributes to circular feed strategies. Modular system design enables adaptation to regional conditions and supports the conversion of local organic side streams into feed resources. These findings provide practical insights into the deployment of BSF systems within European agri-food value chains.

INSECT PRODUCTION, ENGINEERING, AND PROCESSING

Learnings from a decade of insect processing: processing as one key driver for profitability

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Over the past decade, industrial insect farming has developed from experimental pilot systems to commercial-scale production facilities. However, many projects still face challenges in achieving stable economic performance. Industrial experience indicates that biological production efficiency alone is insufficient to ensure profitability. Downstream processing design increasingly emerges as a decisive factor influencing plant economics. This contribution analyses observations from several industrial insect production projects implemented between 2015 and 2025 involving commercial-scale production facilities. Experiences from plant design, commissioning, and early operational phases were evaluated with respect to processing configuration, product strategy, and integration of processing units. Particular attention was given to differences between facilities designed primarily for the sale of unprocessed insect biomass and plants integrating processing steps such as drying, oil extraction, fractionation, and product specification. Across the analysed projects, facilities focused on the sale of unprocessed insect biomass showed significantly higher exposure to market price volatility and margin pressure. In several cases, attempts to introduce processing capacity after plant start-up increased capital expenditure by more than 25% due to retrofitting constraints and suboptimal process integration. In contrast, facilities where processing concepts were defined during early plant design achieved improved process integration, multiple product streams, and more stable product specifications, enabling access to higher-value market segments. The observations indicate that delayed integration of processing systems can lead to structural inefficiencies that are difficult to correct during later plant operation. Conversely, early integration of processing enables better alignment of production systems with downstream product requirements and market demands. In conclusion, the observations confirm that downstream processing is not merely an auxiliary step in insect production but a key driver of profitability. Industrial insect production facilities therefore benefit from being conceptualized as integrated protein processing systems from the earliest project stages in order to improve long-term economic performance.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Host-dominant degradation of sulfonamides in black soldier fly larvae mediated by CYP4d14

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Antibiotic residues in livestock manure, particularly sulfonamides (SAs), pose environmental risks. Black soldier fly larvae (BSFL) are effective in waste valorisation. However, the relative contributions of the larval host versus the gut microbiome to this degradation process remain unclear. This study systematically elucidates the degradation mechanism of Sulfadiazine (SD) and Sulfamethazine (SMZ) from phenotypic, physiological, and molecular perspectives. We established conventional, sterile, and isolated gut microbiome systems to determine degradation contributions. Comparative transcriptomics and molecular docking were used to screen candidate genes. Finally, mechanism validation was conducted using the P450 inhibitor 1-aminobenzotriazole (1-ABT) and functional verification via RNA interference. Phenotypic assays showed that BSFL possesses robust degradation capacity in conventional environments, with removal rates reaching 88.11% for SD and 80.79% for SMZ. Crucially, the sterile BSFL system maintained high degradation efficiency of 72.18% for SD and 85.72% for SMZ, whereas the isolated gut microbiome showed minimal activity ranging from 3.32% to 17.57%, confirming that the larval host rather than the microbiota is the primary driver. Transcriptomic analysis identified the upregulation of Cytochrome P450 genes. This was physiologically validated by adding the inhibitor 1-ABT, which suppressed SD and SMZ degradation rates to 63.17% and 46.66%, respectively. Further molecular docking and RNAi assays pinpointed CYP4d14 as the key gene. Silencing CYP4d14 significantly reduced the degradation efficiency of SD and SMZ to 53.64% and 51.42%, respectively, compared to the control group, which maintained higher rates of 86.67% and 94.38%. This study establishes a comprehensive evidence chain: BSFL degrades sulfonamides primarily through its intrinsic metabolic system, specifically mediated by the CYP4d14 enzyme, rather than gut bacteria. These findings provide a theoretical basis for genetic screening or inducing specific P450 activities to enhance the safety and efficiency of insect-based waste management.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

$CaCO_3$ in shrimp residue inhibits bioconversion and development of black soldier fly (*Hermetia illucens*, Linnaeus, 1758)

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Worldwide population growth has increased the need for sustainable waste management within food systems. Black soldier fly larvae (BSFL) can upcycle various organic residues by converting them into fertiliser (frass) and protein-rich animal feed (larvae). Shrimp processing co-products could be valorised in this way. However, preliminary trials suggested that their high $CaCO_3$ contents could inhibit BSFL growth and bioconversion. This project thus aimed to evaluate the impacts of shrimp co-products and $CaCO_3$ on bioconversion and lifecycle indicators through feeding trials (3 trials; 2 repetitions/diet) using BSFL (4 days post-hatching; n = 1000 larvae/container; 30 mg/ind. per day). We hypothesized that increasing $CaCO_3$ concentration, with or without shrimp products, would diminish growth and bioconversion, and delay development. A control diet (Gainesville, 70% RH) was compared to 9 experimental diets supplemented with raw and hydrolysed shrimp powder (10, 20 and 40%, dry basis) or with $CaCO_3$ (3.3, 4.3 and 8.3% Ca^{2+}, dry basis). Calcium did not accumulate in frass as levels were found to be similar to initial diet levels. In contrast, higher levels of Ca^{2+} were measured in BSFL reared on diets containing 8.3% calcium ($CaCO_3$ supplementation), 40% raw shrimp powder and 40% hydrolysed shrimp powder (from 5.8 ± 0.4% to 15.2 ± 1.0%, 18.7 ± 1.8% and 17.2 ± 0.1%, respectively). Lower bioconversion rates were observed in $CaCO_3$-enriched diets containing 4.3 and 8.3% calcium (from 11.6 ± 0.3% to 5.0 ± 0.5% and 2.8 ± 0.5%, respectively) and 40% raw and hydrolysed shrimp powder (from 11.6 ± 0.3% to 3.7 ± 1.7% and 4.5 ± 0.3% respectively). The CaCO3-enriched diet containing 8.3% calcium also delayed adult emergence (from 29.8 ± 0.3 to 36.5 ± 2.0 days post-hatching). These results indicate that diets containing more than 8% calcium exceed the regulation mechanisms of BSFL, resulting in limited bioconversion and delayed development. Though physiological mechanisms of disturbance (e.g., osmotic stress, ion transport disorders, limiting sequestration capacity of the midgut and Malpighian tubules) require further studies in BSFL, the use of chelating agents could be a promising avenue to enable the upcycling of residues with high calcium content (e.g., marine residues, digestate, milk processing coproducts) through BSFL bioconversion.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Carbon balance and emission reduction in BSF organics conversion significantly enhanced by microbial supplementation

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Global organic waste generation, projected to exceed 6 million tons daily by 2050, drives severe pollution and greenhouse gas emissions. Conventional waste management methods, such as landfilling and composting, contribute significantly to these issues through nitrogen loss and methane release. Black soldier fly larvae bioconversion has emerged as a sustainable alternative that converts waste into valuable protein and biofertiliser. This study employed ^{13}C-labelled sodium carbonate ($Na_2{}^{13}CO_3$) to trace carbon dynamics during BSFL bioconversion of artificial feed, with and without supplementation of the functional microorganism *Stenotrophomonas acidaminiphila* (CF-5). Carbon emissions were quantified using absorption and multi-gas systems, and microbial community dynamics were profiled via 16S rRNA sequencing and ^{13}C-DNA-based stable isotope probing. The results showed that BSFL conversion alone reduced cumulative CO_2 emissions by 23.37% compared to natural fermentation over 51 days. CF-5 supplementation further reduced emissions by 13–13.38% and suppressed system carbon loss by approximately 75%. Isotopic tracing revealed that CF-5 enhanced ^{13}C assimilation into larval biomass (increasing carbon content by 11–28%), with significant enrichment in fat bodies and exuviae, while concurrently decreasing $^{13}CO_2$ release. Microbial analysis indicated that CF-5 enriched Actinobacteria in BSFL guts and upregulated key carbon fixation pathways — including the Calvin–Benson–Bassham cycle in the substrate and the Wood-Ljungdahl, rTCA, and 3HB pathways in the guts — without altering overall microbial diversity. The results indicated that functional microorganisms such as CF-5 optimise carbon flux in BSFL systems, augmenting carbon sequestration by redirecting waste-derived carbon into larval biomass. This approach establishes an efficient strategy for carbon-neutral organic waste management.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Valorisation of challenging waste streams using *Hermetia illucens* for animal feed and human food

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Black soldier fly larvae (BSFL) are increasingly recognised as an efficient and sustainable tool for converting organic waste streams into high-quality protein for animal feed and potential human food. However, many of the abundant food-chain wastes used as feeding substrates (e.g., grape marc (GM), olive pomace (OP), paunch/offal, pre-consumer food waste) are considered "challenging" due to high lipid, phenolic, fibre, or salt content, which can impair larval growth and survival. These substrates may also contain contaminant residues that implicate the final BSFL safety. This study investigated the performance and safety of BSFL reared on 11 real-world, complex substrates that are currently under-investigated due to these technical challenges. Neonates from commercial Australian colonies were reared to maturity on the following substrates: mixed fruit/vegetable, GM, OP, fermented banana skins, creamed corn, dog food or cattle paunch/offal mixture. Larval growth, substrate reduction, bioconversion rate, and final biomass yield were recorded. Proximate composition and fatty acid profiles of BSFL were determined, with >300 pesticide residues (including glyphosate, cyprodinil, and metalaxyl) monitored in substrate, larvae and frass. BSFL achieved good performance on most substrates: growth 49–178 mg/larvae; waste reduction 52.9–78.5% (w/w); and feed conversion ratio 14.7–22.4 dry matter (DM). Grape marc and olive pomace at 50% inclusion, traditionally considered difficult, supported larval final weights of 106 ± 6 mg and 109 ± 6 mg (fresh), respectively, with protein content 38–43% and fat 26–35% DM. Larval biomass contained favourable ω-6/ω-3 ratios <8:1 in several treatments. Pesticide carry-over was compound-specific: metalaxyl was eliminated, cyprodinil was quantitatively transferred to frass, and glyphosate showed partial uptake (10–43% of substrate concentration as dry matter) with significant excretion. Highly toxic compounds such as paraquat accumulated in some BSFL protein fractions, highlighting the need for substrate screening. BSFL demonstrated remarkable adaptability to high-phenolic GM, high-lipid OP, and high-sodium (paunch and dog food) substrates, producing nutritionally valuable protein meals with quantitative performance comparable to fruit and vegetable substrates. Pesticide fate was predominantly favourable, with strong reduction or excretion for most compounds, confirming the bioremediation potential of BSFL. These results support the safe upscale of BSFL technology for valorising challenging food-chain side streams that are currently landfilled, contributing to circular economy and sustainable feed and food production.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Integrating sustainability assessment and advanced analytics in insect farming

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Insect farming is increasingly recognised as a promising pathway for sustainable food and feed production, yet its effective scaling is constrained by fragmented analytical approaches and limited integration of sustainability assessment with advanced data analytics. While environmental, economic, and social impacts are routinely discussed, there is a lack of unified methodological frameworks capable of supporting robust evaluation, optimisation, and data-driven decision-making in research, industrial and policy contexts. This gap reduces comparability between studies, limits reproducibility, and weakens the evidence base required for large-scale deployment and regulatory acceptance. This study synthesises current analytical practices in insect farming, with a specific focus on the integration of sustainability assessment tools and advanced data analysis methods. Sustainability evaluations based on life cycle assessment (LCA), life cycle costing (LCC), and social life cycle assessment (S-LCA) are reviewed as key instruments for capturing environmental performance, economic viability, and social implications of insect-based systems. In parallel, there is an obvious development of advanced analytics, including decision support systems, bioeconomic models, machine learning techniques, and automated monitoring tools, to optimise production efficiency, resource use, behaviour analysis, and selective breeding. Even though there are advances in both fields, their cross-application is limited. Advanced analytics has a strong potential to improve not only operational efficiency and predictive capacity but also automate and standardise sustainability assessment if assured with good data quality and interoperability. It can solve the issues of methodologic heterogeneity and inconsistency in reporting practices of sustainability assessment. Furthermore, emerging digital technologies, including blockchain and predictive analytics, offer additional opportunities to enhance traceability, regulatory compliance, and food safety assurance in automated regime. Overall, the integration of harmonised sustainability assessment frameworks with advanced analytical tools is critical for advancing insect farming from experimental systems to scalable, resilient, and compliant production models. Establishing unified guidelines, improving data infrastructures, and aligning analytical methods with regulatory and industry needs will be essential to unlock the full sustainability potential of insect-based food and feed systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Ingestion of polyurethane foam by *Tenebrio molitor* larvae: effects of nutritional and probiotic supplementation

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Insect species such as *Tenebrio molitor* have been proposed as an alternative to mitigate the environmental impact of plastic waste. A 28-day experiment was conducted to evaluate the ingestion of polyurethane foam by *T. molitor* larvae and to assess their ability to use it as an energy source when supplemented with a nutritional substrate and a bacterial probiotic mixture. Eighteen trays containing 60 larvae (average initial weight: 25.3 mg) were assigned to three treatments, in which polyurethane foam was offered *ad libitum* as the sole substrate (PU) or supplemented with wheat bran (0.4 g per week) either without (PUB) or with a mixed bacterial culture (*Kocuria rhizophila*, *Priestia megaterium* and *Cupriavidus respiraculi*) administered as a probiotic (PUBP). Fresh carrot was provided twice a week as a source of water. Despite the limited nutritional value of polyurethane, larval mortality was low (average 7.2%) and did not differ among treatments. Larval growth and total mass produced were greater in PUB and PUBP than in PU ($P < 0.001$), although numerical improvements observed in PUBP compared to PUB were not statistically significant. Polyurethane intake reached 9.85 mg/larva and did not differ among treatments, whereas frass production was higher in PUBP than in PU, with intermediate values observed in PUB ($P < 0.001$). Microscopic examination of frass revealed the presence of numerous polyurethane particles across all treatments, indicating incomplete polymer degradation. In conclusion, *T. molitor* larvae were able to ingest polyurethane foam independently of additional nutrient supplementation. The provision of small amounts of wheat bran improved larval performance without affecting plastic intake. Although larval depolymerising capacity and the potential contribution of probiotic supplementation could not be conclusively demonstrated under the present experimental conditions, the numerical improvements in productive performance observed in probiotic-supplemented larvae suggest that a potential enhancement of plastic degradation cannot be completely discarded.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Monitoring and manipulating the microbiota of yellow mealworm larvae (YML) to improve their bioconversion performance

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Tenebrio molitor (YML) offers promising solutions for organic waste upcycling into protein and biofertiliser. However, upcycling waste is complicated by its instability, variability and digestibility. Therefore, this project seeks to implement a microbiota-centric approach to improve bioconversion performance and reduce the environmental footprint of waste upcycling bioprocess. Considering the central role of microbes for insect host and feedstock stability, we hypothesized that bioconversion performance is related to microbial community structure and functioning in feedstock and YML. The hypothesis is tested by longitudinal monitoring of YML farm and laboratory-scale experiments. The farm-scale experiment began in June 2025. YML conversion rate, mortality, farm temperature and microbiome of feedstock, larvae and frass are monitored to relate microbiome and environmental conditions to farm productivity. Microbial taxa whose distribution is beneficial or deleterious to YML productivity will be identified and directed isolation procedures will be deployed to demonstrate how their introduction in the YML affects biomass production yield. While farm monitoring efforts continue until June 2026, preliminary data assess variation of microbiome composition, potential functions and diversity in YML, frass and feedstock. These observations also support the second, lab-scale experiment seeking to manipulate the microbiota of wet feedstock and YML for better yield. The lab-scale experiment aimed to improve the shelf-life of wet diet by lactic-acid producing bacteria (LAB) fermentation. Besides feedstock stabilization, few LAB strains were shown to act as probiotics in YML. A collection of LAB associated with YML was assembled, and isolates were subjected to DNA extraction and 16S rRNA gene PCR for molecular identification. Isolates were screened for their fermentation performance using fruit-vegetable mixture from waste management industry. Around 50 colonies were isolated from YML under anaerobic conditions, mainly representing the genera *Lactococcus*, *Enterococcus*, *Staphylococcus*, *Weissella* and *Brevibacterium*. Preliminary results reporting the feedstock fermentation impact for 1,7 or 14 days on YML growth performance and feedstock quality will be presented. In conclusion, this communication is expected to give rise to inspiring exchanges about the challenges and promises of microbiological applications in ento-technologies to improve organic waste upcycling.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

A modular decision support system (DSS) for cost and environmental optimisation in insect production

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Insect-based protein production offers a potential for reducing the environmental footprint of food and feed systems while providing protein-rich outputs. However, large-scale production is often constrained by high feed and processing costs, inconsistent nutritional contents, and processing-related environmental impacts. This study presents the development of a data-driven decision support system (DSS) to assist insect producers in minimising production costs and environmental impacts while maintaining higher protein content and potential financial gains. The DSS integrates experimental performance data, feed costs and process-level resource use into a modular optimisation framework. Feed requirements and costs per unit of dried larval biomass are estimated using feed conversion ratios and biomass yields, including mixtures of multiple feed types to identify cost-efficient combinations with high protein output. Environmental impacts are modelled across the production chain, distinguishing feed-related effects from processing inputs (electricity, heat, water and land) in relation to country and insect species. Cost and environmental indicators are combined using multi-criteria optimisation, including constraint-based filtering and Pareto-front analysis, supported by linear programming and heuristic optimisation methods depending on data availability. In a preliminary analysis of five feed types from Greece, the cost and environmental impacts of producing 1 kg of yellow mealworm dry larval biomass were evaluated using blanching and oven drying as the standard processing route. A feed mixture of oats by-products (65%) and maize by-products (35%) resulted in the lowest carbon emissions (0.122 kg CO_2-eq), representing a 75% reduction compared to the mean emission value (0.474 kg CO_2-eq). The estimated feed cost for this mixture was approximately €0.61 per kg of dry larvae, excluding processing costs. DSS demonstrates how heterogeneous experimental data can be translated into an actionable tool for sustainable insect production. By integrating cost modelling, environmental assessment, and optimisation logic within a software-driven framework, the DSS provides a practical foundation for informed decision making in insect-based production systems and is being implemented as a web-based application for stakeholder use.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Field evaluation of black soldier fly frass as an organic fertiliser for maize production in southeastern Madagascar

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Food insecurity remains a major challenge for smallholder farming communities in Madagascar, underscoring the need for locally available and sustainable soil fertility inputs. Black soldier fly frass (BSFF), a by-product of insect-based organic waste conversion systems, is an emerging organic fertiliser; however, field-based evidence of its agronomic performance under smallholder conditions remains limited. This study evaluated the effects of composted BSFF (CBSFF) and fresh BSFF (FBSFF) on maize (*Zea mays* L.) growth and yield in comparison with cattle manure (CM) and an unfertilised control in acidic sandy clay soils of southeastern Madagascar. A field experiment was conducted using a nitrogen application rate of 43 kg N ha^{-1}, corresponding to approximately one-third of the national recommendation and reflecting prevailing farmer practice in the region. Germination rate, plant height, grain yield, and agronomic efficiency of nitrogen (AE_N) were assessed. Maize treated with FBSFF exhibited significantly lower germination rates than all other treatments, indicating sensitivity to application timing. No statistically significant differences in final plant height or grain yield were observed between CBSFF and CM; however, plants fertilised with CBSFF were on average 6% taller than those receiving CM and 13% taller than those receiving FBSFF. Grain yield under CBSFF was 38% higher than under CM. All fertilised treatments produced significantly greater yields than the unfertilised control, which resulted in no harvestable maize. CBSFF showed the highest agronomic efficiency of nitrogen (46 kg grain per kg N), although differences among fertiliser treatments were not statistically significant. Overall, results indicate that BSFF, particularly in composted form, can supply sufficient nitrogen to support maize production under smallholder conditions. Composted BSFF appears more suitable for pre-planting application, while fresh BSFF may be better applied after crop establishment. Even at sub-recommended fertiliser rates, BSFF demonstrates potential to enhance maize productivity within circular, insect-based waste management systems and contribute to local food security. Further research across multiple agroecological zones, including economic analyses of cost, labor, and transport, is recommended to support broader adoption.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

A review on insects and urban circularity: future pathways for food loss and waste interventions

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Over one-third of food produced for consumption is either lost or wasted in the United Kingdom (UK), which is 25% of edible food costing the economy over £20 billion. Meanwhile, a significant percentage of the population experiences food insecurity and hunger across the globe. To avoid these circumstances, as policy attention gradually shifted towards circular economy explanations, such as simpler recycling legislation in the UK, voices advocating for alternative interventions for organic waste valorisation and sustainable food system transitions likewise amplified. Therefore, this study investigates the role of insects as a circular bio-solution in the city value chain and design, examining their potential impact on organic waste interventions in the UK. Drawing on food system theory and city-region perspectives, this study assesses the insect-based interventions as an integrated unit for managing FLW, where organic waste from metropolitan areas is considered a resource and transformed into valuable produce for both urban and rural adoption needs. All these steps are carried out by reviewing literature on FLW, unpacking policy, practice, Life Cycle Assessment, circularity, insect bio-conversion and valorisation, towards determining key enablers, players, barriers, pathways and opportunities for adoption. Findings highlight the potential of insect-based approaches in rethinking urban organic waste design and propose a new "insect city approach" to decentralising FLW and organic waste, often mismanaged and a missed opportunity for sustainable food systems, while also revealing key policy drawbacks and green investment prospects. This research argues that green spaces in cities are vital spaces for insect bio-solution take-off and integration into FLW interventions, hence the need for a robust policy framework and implementation. By reframing insects as enablers of city circularity, this study contributes to debates on urban design and circular food systems and provides insights for various stakeholders exploring innovative pathways to integrate FLW management into urban food systems and shift the burden of FLW from waste redistribution companies.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Recirculating BSF frass for an optimised waste bioconversion and high-quality fertiliser production

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The use of drying agents when treating waste with *Hermetia illucens* larvae is a common industrial practice that increases costs and affects the activity's environmental performance. Frass recirculation, in which frass is used as a dietary component while simultaneously serving as a drying agent, has been proposed as an alternative. In this study, frass recirculation (15-25% inclusion level) was studied for various organic wastes — food waste, spent grains, pig manure, fruits and expired chicken feed — with initial DM contents of 29, 24, 22, 13 and 46%, respectively, which after a first cycle of bioconversion generated frass with DM contents ranging between 46 and 75%. Frass obtained from recirculation cycles were analysed for their maturity (pH, electrical conductivity, seed germination) and composition (plant nutrients, microorganisms and phytohormones). Frass collected from a bioconversion with four recirculation cycles were used as fertilisers for cultivating pak choi (*Brassica rapa*), and its effects on plant growth and photosynthetic performance were assessed. Recirculation appeared viable for most of the tested substrates, achieving good process performance in relation to larval yield (comparable with the controls, without recirculation). The exception was spent grains, likely due to the high bulkiness of the generated frass, which increased substrate depth. Recirculating frass at least once resulted in increased maturity of the fertiliser in comparison to fresh (non-recirculated) frass, with seed germination indexes being up to 10-fold higher in recirculated frass. Recirculation was found to shift the frass' microbial community. Firmicutes and Proteobacteria were more prominent in recirculated frass, while Actinobacteria was more prominent in fresh frass. Specific phytohormones were screened in frass samples, such as cytokinins (up to 70 pmol (g DM)$^{-1}$) and auxins (up to 1050 nmol (g DM)$^{-1}$), positively correlated with humic substances. Specific bacteria (e.g. *Oceanobacillus*, *Cerasibacillus* and *Morganella*) positively correlated with phytohormones, suggesting that the biostimulant effects of frass may be derived from its microbial composition. Pak choi plants fertilised with recirculated frass reached similar fresh weight (241 ± 19 g fresh weight) in comparison to control plants (232 ± 44 g) cultivated with a chemical fertiliser, while achieving 5-15% higher photosynthetic efficiency. Frass recirculation is a promising alternative to reduce the need for drying agents, resulting in positive effects on process efficiency and frass fertilising quality.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Impact of predigestion of organic residues on black soldier fly protein and its use in organic post-weaning pigs

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Black soldier fly (BSF) *Hermetia illucens* larvae offer a robust option to upcycle food loss and waste into high-quality proteins for animal nutrition in circular and organic systems. Within the ASTIPPOR project, we established in Wallonia a pipeline that predigests local organic food surpluses and brewers' spent grains using a thermophilic aerobic digester, rears BSF larvae on the stabilized substrate, and valorises defatted BSF meal in post-weaning piglet diets under organic conditions. We first assessed five physical and microbial pretreatments (grinding, heating at 50 °C, and thermophilic microbial inoculation, alone or combined) of a mixture of unsold fruits and vegetables, bread, and brewers' spent grains on BSF performance and composition. All larval groups reached high final weights (0.278–0.307 g at day 12; 0.326 g on chicken feed) with survival rates above 92%, while predigestion significantly altered larval lipid content and fatty acid profile but not protein or ash content (≈30–31% crude protein DM, ≈32% lipids DM, ≈3.5% ash DM). Predigested substrates produced larvae with lower total lipid and saturated fatty acid proportions and slightly higher unsaturated fatty acids, improving the protein-to-lipid ratio for monogastric diets. Defatted BSF meal was then used to partially replace the protein core (soybean meal, pea meal, potato protein) in organic post-weaning piglet diets. In a five-week trial with 72 female piglets, four isoenergetic and isonitrogenous diets were tested: a control without insects and three diets where 15, 25, or 35% of the protein core was replaced by defatted BSF meal. At 15% replacement, growth performance, weekly gain, body-weight trajectories and feed conversion ratio did not differ from the control, whereas higher inclusion levels caused modest but significant increases in FCR and slightly lower final body weights. Cumulative FCR remained within typical organic ranges, but economic modelling showed that the break-even price of insect meal rapidly declines when inclusion exceeds 15% of the protein core, constraining economic viability at current market prices. Overall, ASTIPPOR demonstrates that thermophilic microbial and physical predigestion of local organic residues can generate BSF larvae with a nutritionally favourable profile, and that defatted BSF meal can safely replace up to 15% of the protein core in organic post-weaning piglet diets without impairing performance.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Upcycling regional fruit and vegetable by-products into eco-feed using black soldier fly (*Hermetia illucens*)

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Globally, the black soldier fly (*Hermetia illucens*, BSF) has emerged as a major insect species for organic waste bioconversion, supported by increasing industrial deployment in feed and waste-processing sectors. In South Korea, the first-tier sales value of BSF increased from KRW 0.8 billion in 2017 to KRW 1.09 billion in 2021, accompanied by an increase in the number of BSF farms from 166 to 224, indicating rapid expansion of on-farm production capacity based on national insect industry statistics. This study quantitatively evaluated the bioconversion performance of BSF larvae fed regional fruit and vegetable by-products, focusing on waste reduction efficiency, biomass conversion, and key biological parameters directly relevant to industrial eco-feed utilisation. BSF larvae were reared on apple pomace, mandarin waste, and oriental melon waste under controlled conditions (26 °C, 60% RH), and larval growth, developmental time, survival, fecundity, waste reduction ratio, and bioconversion efficiency were measured. Larvae decomposed 48.0% of apple pomace and 61.5% of mandarin waste, yielding bioconversion efficiencies of 9.1% and 12.1%, respectively, with no significant differences in larval survival (≥88%) or adult fecundity (≥99%) compared with a commercial control diet. When fed oriental melon waste, BSF larvae achieved a substantially higher waste reduction rate of 80.5% and a bioconversion efficiency of 11.8%, although larval development was prolonged and individual larval biomass was reduced relative to nutrient-balanced feeds. Across substrates, waste reduction efficiency was strongly associated with substrate physical properties (semi-liquid texture, low fibre content), whereas larval biomass accumulation was primarily constrained by protein and lipid availability. These results demonstrate that region-specific horticultural by-products can be reliably processed by BSF larvae with predictable trade-offs between waste reduction efficiency and larval biomass yield. The quantitative outcomes provide a technical basis for (i) substrate-specific precision rearing strategies, (ii) selective breeding targeting improved conversion efficiency under low-protein substrates, and (iii) optimisation of reproductive performance for large-scale BSF eco-feed system integration.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Treatment of food processing wastewaters by using black soldier fly larvae

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The use of black soldier fly larvae (BSFL) is a promising alternative for treating high-organic-content (HOC) wastewaters (WWs), with high efficiency and the production of valuable biomass. Although widely applied to solid biowaste, their use in wastewater is limited by drowning. This was overcome by providing inert solid support (plastic granules) saturated with wastewater, allowing larvae to dive to feed and resurface to breathe. This process is termed LarWaR (Larvae for Wastewater treatment and Resource recovery). This study evaluates LarWaR on six food-processing WWs: bakery (BA), brewery (BR), dairy (D), juice (J), slaughterhouse (S) and winery (W). Suitability was assessed based on organic content and macronutrient composition (protein, non-fibre carbohydrates (NFC), lipids). Performance was evaluated in batch tests by monitoring larval growth, mortality, and removal of organic carbon, nitrogen, and phosphorus. Each reactor (13.5 × 13.5 × 5.5 cm) contained 80 six-day-old larvae (8.4 mg wet weight, SE = 1.08; density 0.4 larvae/cm^2) and a plastic granular bed (VALOX, 2 – 3 mm) saturated with 150 ml WW. Reactors were covered with permeable fabric and perforated lids to allow aeration and prevent escape or oviposition. WW was replaced weekly for 49 days. WWs differed in organic concentrations, resulting in different applied loads. Each WW was tested in triplicate (21 reactors total) at 25 – 30 °C, 18/6 h light/dark. All WWs achieved TOC removal of 76 – 93%, BOD 86 – 97% and COD 75 – 85%. Concentrations halved within 2 weeks and stabilized by 3 weeks, indicating a short start-up compared to conventional treatments. The specific consumption rate (mg C/larva per day) increased proportionally with organic load, following a Michaelis – Menten relationship, while removal efficiency decreased with increasing load. This relationship can be applied to design LarWaR systems and predict performance across HOC WWs. Considering both treatment and larval development, BA, BR and W (TOC > 1500 mg C/l; load 0.4 – 0.55 mg C/larva per day) performed the best, with the lowest mortality (<20%) and the highest larval weight (109 mg, SE = 4.5). These WWs had higher levels of volatile fatty acids (VFAs) and ethanol than NFC, suggesting that larvae used them as additional carbon sources, thereby improving macronutrient balance and performance. Although LarWaR effectively removes organics, nitrogen and phosphorus were only partially removed, indicating the need for additional treatment steps to address these contaminants. Results highlight the importance of organic concentration and macronutrient quality, which can be optimized by mixing WWs to enhance performance and applicability.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Gut immune responses in black soldier fly larvae: the role of the gut microbiota to prime pathogen resistance

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Organic substrates used for rearing black soldier fly larvae (BSFL) may harbour pathogenic bacteria, such as *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Bacillus cereus*, posing potential risks to both human health and animal welfare. Nevertheless, BSFL thrive in pathogen-rich environments and can significantly reduce bacterial loads in the rearing substrate, suggesting the presence of a highly effective immune system. While the cellular and molecular mechanisms underlying systemic immune responses to infections have been partially characterised, the contribution of gut microbiota and gut physiology to pathogen defence remains poorly understood. To investigate the role of the gut microbiota as a factor for immune stimulation in the defence mechanisms of BSF, we adopted two complementary challenges to assess changes in host immunity within an axenic BSFL rearing system: (i) *S. aureus* and *E. coli* were inoculated in the rearing substrate (7 log CFU/g), to assess pathogen resistance in the gut environment and (ii) the same microbes were injected into the hemocoel (7 log CFU/ml) to assess pathogen resistance within the larvae. Challenged axenic larvae exhibited higher survival rates following the direct injection than control, while gut microbial loads were comparable to those of controls after the dietary challenge, indicating that gut microbiota is not essential for the activation of BSFL immune defences. Larvae remain, indeed, immunologically primed despite the absence of a gut microbial community. However, while control larvae significantly reduced bacterial loads in the substrate after the dietary challenge, pathogen levels remained high under axenic conditions, highlighting the importance of interactions between BSFL and the environmental microbial community in pathogen suppression. The gut immune response will be further analysed to verify that no underlying shifts in the activity of immune effectors have occurred. This study provides new insights into the complex interplay between host immunity, gut microbiota, and environmental microbes in BSFL. Such insights offer a foundation for future research that aims to develop strategies that enhance larval immune competence, with potential applications in improving biosafety, resilience and sustainability in large-scale waste bioconversion systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Well-timed provision of digestible carbohydrates improves manure utilisation by housefly larvae

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Rearing housefly larvae (*Musca domestica* L.) on livestock manure, such as chicken excreta, can contribute to sustainable feed production and waste reduction. As chicken excreta contains low amounts of digestible carbohydrates, adding these could enhance larval biomass and nitrogen retention. However, ontogenetic changes in morphology and enzymology indicate that larval use of carbohydrates may differ over larval stages. This study tested how carbohydrate type (glucose, starch, cellulose, fresh excreta as control) and timing of supplementation (0 h or 72 h) affects larval growth, bioconversion and residue characteristics. Glucose and starch improved larval performance compared to fresh manure and cellulose. Late (72 h) supplementation was most effective, with late glucose increasing individual larval mass by 12%, total biomass by 33% and survival by 19%. Late starch increased total biomass by 30% and survival by 24%, while effects on individual mass were small (2% increase). Dry matter bioconversion efficiency increased by 27% with late glucose and 20% with late starch, whereas late excreta addition caused an 18% decline. Nitrogen bioconversion efficiency rose by 36% with late glucose and by 37% with late starch. Residue ammonia increased with all supplementations but less for with late additions than with early additions, especially for starch. Residue pH increased with all supplementations, with excreta and cellulose showing the largest early-late increases (0.3-0.8), early and late glucose numerically decreasing and late starch producing the overall lowest pH. This study shows that adding starch to fresh chicken manure later in the larval development period improves survival, bioconversion and yield.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Enabling waste-based insect feed systems for circular agri-food transformation: governance lessons from Uganda

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Insects reared on organic waste streams offer a promising pathway for advancing circular agri-food systems by converting waste into high-quality animal feed while reducing environmental pressures. In low- and middle-income countries, waste-based insect feed systems are particularly relevant due to high feed costs, widespread organic waste mismanagement, and growing demand for sustainable livestock production. Despite strong technical and agronomic evidence, adoption and scaling of insect-based feed systems remain limited. This paper examines how policy, legal, regulatory, and institutional frameworks shape the emergence and scaling of waste-based insect feed systems in Uganda, with complementary insights from waste-derived organic fertiliser pathways. The study combines a systematic review of national policies, laws, regulations, and standards governing waste management, animal feed, and fertilisers with a stakeholder power-influence analysis to explore the political economy of implementation. Findings reveal strong alignment between insect-based circular economy principles and national objectives on waste reduction, livestock productivity, and soil health. However, regulatory ambiguity around the classification of organic waste and waste-derived insect feed, fragmented institutional mandates, and centralized compliance systems constrain operationalization. High compliance costs and procedural complexity disproportionately exclude micro, small and medium enterprises, which dominate insect production and innovation. Power-influence asymmetries further reinforce regulatory inertia, as actors with practical expertise in insect rearing and waste valorisation have limited influence over rule-making processes. The paper demonstrates that regulatory clarity, institutional coordination, and inclusive governance are critical for unlocking the scaling potential of waste-based insect feed systems and advancing circular agri-food transformation.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Synthesising the evidence on *Salmonella* spp. inactivation in black soldier fly larvae bioconversion of biowastes

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Insect bioconversion has been recognised as a circular waste management method and protein production strategy. Life cycle assessments show that environmental benefits require the use of "true" waste substrates. Post-consumer food waste may contain pathogenic microorganisms, including zoonotic *Salmonella* spp. At the Environmental Engineering group at SLU, we have studied *Salmonella* inactivation during black soldier fly larvae (BSFL) bioconversion since 2013 and consistently observed inactivation (Lalander *et al.*, 2013, 2015). The frequency of contaminated feed influenced reduction (Lopes *et al.*, 2020), with lower total reduction (3.3 $\log_{10}$) under multiple feedings compared to a single feeding (6.2 $\log_{10}$). Frass alone also inactivated *Salmonella* when reinoculated without larvae, suggesting antimicrobial activity. In a later study (Lalander *et al.*, 2025), neither treatment scale (2400 vs 231 cm^2) nor 20% frass inclusion affected inactivation, despite the hypothesis that frass would enhance it. In our most recent work, we examined larval density (1, 3, 6 larvae/cm^2; 0.6, 0.2, 0.1 g VS/larva) and substrate composition (carbohydrate-rich (CHO) vs protein-rich (Pr) diets). In the CHO diet, reduction appeared mainly chemical, as similar reductions occurred in controls; this was not observed in the Pr diet. Under repeated contamination, inactivation occurred only in the Pr diet and decreased at higher densities. At high densities, larval weights were <150 mg/larva compared to 165–200 mg/larva at lower densities, indicating suboptimal growth. The Pr diet had similar crude protein (approx. 30% DM) but lower crude fat (12% vs approx. 30% DM; Lopes *et al.*, 2024). It could be speculated that reduced inactivation at higher densities may be linked to nutrient or energy deficiency. Lalander, C., Diener, S., Magri, M.E., Zurbrügg, C., Lindström, A., and Vinnerås, B., 2013. Faecal sludge management with the larvae of the black soldier fly (*Hermetia illucens*) – from a hygiene aspect. Science of the Total Environment 458-460: 312-318. Lalander, C., Fidjeland, J., Diener, S., Eriksson, S. and Vinnerås, B., 2015. High waste-to-biomass conversion and efficient *Salmonella* spp. reduction using black soldier fly for waste recycling. Agronomy for Sustainable Development 35: 261-271. Lalander, C., Lopes, I.G., Gyftopoulos, N. and Vinnerås, B., 2025. The impact of scale and frass recirculation on pathogen inactivation dynamics in black soldier fly larvae bioconversion. Frontiers in Microbiology 16: 153948. Lopes, I.G., Lalander, C., Vidotti, R.M. and Vinnerås, B., 2020. Reduction of bacteria in relation to feeding regimes when treating aquaculture waste in fly larvae composting. Frontiers in Microbiology 11: 1616. Lopes, I.G., Wiklicky, V., Vinnerås, B., Yong, J.W.H. and Lalander, C. 2024. Recirculating frass from food waste bioconversion using black soldier fly larvae: Impacts on process efficiency and product quality. Journal of Environmentl Management 366: 121869.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Valorisation of school canteen food waste with black soldier fly larvae in Central Europe via decentralised insect farms

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Food waste (FW) from school canteens is an underutilised resource that can be valorised through insect bioconversion, support circular food systems and local education. Because school FW production is decentralized, we investigated how school leftovers could be valorised by decentralized insect bioconversion using black soldier fly larvae (BSFL) within the EU Interreg FoodCIRCUS project. As EU regulations prohibit the use of postconsumer catering waste as insect feed, practical recommendations were formulated. The case study combined three methods. First, FW generation was quantitatively assessed in school canteen across five Central Europe countries. Monitoring surveys covered 23 schools in Austria, 17 in Italy, 6 in Poland, 9 in the Czech Republic and 10 in Hungary. Second, survey data were used to calculate mass flow for integrating FW into a decentralized insect bioconversion system (9500 t substrate/year). Third, representative FW samples collected over four days in schools in Vienna and Leipzig were used as substrates for BSFL. Wheat bran and water were used as a control diet. FW was minced (< 5 mm), adjusted to 73–75% moisture, and portioned into 9 kg batches in 40 x 60 cm boxes. Each batch reared 13 000 7-day-old BSFL for 8 days. Process performance indicators, such as larval yield, bioconversion efficiency, substrate reduction, frass output and characterisation of the chemical and microbiological properties of both larval biomass and frass were assessed. Monitoring in the five FoodCIRCUS partner countries provided a baseline data: Hungary showed the highest share of discarded food (48%) followed by Poland (46%), Austria (44%), the Czech Republic (35.5%), and Italy (29.3%). Austrian schools generated on average 12.6 kg of FW per canteen per day. Extrapolated to 250 Viennese schools, this equals about 3.1 t per day (≈ 567 t per school year). Scenario analysis showed that school FW from larger cities could supply 6% of substrate demand for a decentralized insect farm. The study confirmed that school FW is an efficient BSF substrate. Bioconversion efficiency of FW reached 18%, substrate reduction rate was up to 91%, frass output was lower on FW diets (0.83–0.97 kg vs 1.24 kg in the control), and no Salmonella was detected in the frass. The technical findings were linked to practical constraints such as collection frequency, pre-treatment and hygienisation options, and storage capacity. Despite current legal barriers, integrating school canteen food waste as a substrate material in decentralized insect farming is technically feasible.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Valorisation of olive pomace and other Algerian agro industrial by products in black soldier fly rearing

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Hermetia illucens larvae are increasingly recognized as a sustainable biological agent to convert organic waste into protein-rich biomass and frass within a circular bioeconomy framework. In Mediterranean regions, agro-industrial activities produce substantial by products each year, notably olive pomace, constitutes a significant fraction of these residues. Their use remains limited, underscoring the importance of exploring alternative valorisation strategies. This study evaluated the efficiency of a mixed substrate formulated with 33% olive pomace, 34% brewery spent grain and 33% recycled bread on larval growth, substrate reduction, bioconversion efficiency, and nutritional quality of larval biomass and frass, compared to a standard Gainesville diet as a control. A total of 27 000 5-day-old larvae were allocated into two treatments with three replicates each. Larval growth parameters (weight, length, width) were recorded every two days. At the end of the trial, substrate reduction rate (WR), bioconversion efficiency (BRE), mortality rate (MR), meal yield, and the chemical composition of larval biomass and frass were determined. Larval growth was significantly influenced by substrate type ($p < 0.05$). On day 15, larvae fed the mixed substrate reached a higher average weight (0.233 ± 0.009 g), length (24.23 ± 0.31 mm), and width (5.30 ± 0.06 mm) than those fed the Gainesville diet (0.184 ± 0.006 g; 22.16 ± 0.61 mm; 4.52 ± 0.19 mm). Substrate reduction was significantly higher with the mixed diet (85.40%) than the control (66.40%, $p \leq 0.01$), and bioconversion efficiency was more than twice as high (21.69 ± 0.63% vs 10.05 ± 0.20%). Mortality rates remained very low in both treatments. The chemical composition of larval meal was similar between treatments, with crude protein around 44% and ether extract between 41 and 44%. In summary, incorporating olive pomace even at moderate levels into BSFL diets allows efficient valorisation of local agro-industrial waste enhancing BSFL growth, substrate reduction, and bioconversion efficiency. It produces protein-rich biomass and nutritionally valuable frass, contributing to a sustainable circular bioeconomy. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA Repurposing OLIve Waste in circular economy solutions for feeds, additives, packaging, and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Regulatory latitude and black soldier fly rearing in North America

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Insect, and more specifically black soldier fly (*Hermetia illucens*), rearing is increasingly promoted as a circular solution for organic waste valorisation. However, sustainability performance and economic viability differ substantially across regions, often reflecting regulatory context rather than biological or technical constraints. This study examines how feedstock regulations shape outcomes in insect-based waste valorisation systems. A comparative review of regulatory frameworks was conducted across Europe, the United States, Australia and China, focusing on feedstock eligibility, waste classification, and sustainability objectives. International trade data for insect-derived products were reviewed as an indicator of global production capacity and demand. Substantial regulatory divergence was identified. In the United States, Australia and China, black soldier fly systems are commonly integrated into waste management strategies and may utilise a broad range of organic inputs such as recovered food waste, post-consumer substrates, or manure. In contrast, other regions apply more restrictive feedstock rules, emphasizing precaution and feed safety. Despite these differences, global production continues to expand, with North America importing more than 10 000 tonnes of insect-derived products annually. These results demonstrate that sustainability outcomes are closely linked to regulatory design. Broader access to organic waste streams generally supports higher waste diversion and enhanced circularity, while more restrictive frameworks prioritize traceability and risk management. Both approaches reflect valid policy goals but lead to different operational and environmental results. We conclude that black soldier fly rearing is a flexible technology whose contribution to sustainable waste valorisation depends strongly on regulatory context. Evaluations of insect agriculture should therefore be region-specific. Aligning science-based risk management with circular economy objectives will be critical to maximising environmental benefits.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

EcoeFISHent: Circular valorization of fish by-products through insect bioconversion

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The EcoeFISHent project, funded under Horizon Europe, addresses the challenge of underutilised fish processing by-products by developing and put in action innovative circular solutions. The project explores insect bioconversion as a sustainable pathway to transform these streams into valuable outputs. A dedicated pilot unit running by an Industry-Academia partnership is assessing the technical feasibility of using fish by-products as substrates for insect rearing. While current EU regulations currently restrict the use of certain fish-derived side-streams as insect substrate, EcoeFISHent focuses on research and demonstrate how high-quality fish side-streams can be safely bio converted, paving the way for more direct circular value chains and open the dialogue for future frameworks. Furthermore, the 10 tons annual biomass capacity of the pilot aims to produce frass, chitin/chitosan, and insect oil, unlocking applications beyond feed markets: frass as an organic fertilizer, chitosan for agriculture and pharmaceuticals, and oil for cosmetics. These outputs provide a qualitative demonstration of how insect technologies can complement other circular strategies, creating new value chains aligned with the European Green Deal, EU Bioeconomy Strategy, EU Circular Economy Action Plan and other sectoral or regional sustainability strategic frameworks. EcoeFISHent unites industry, research and technology partners in the first regionally defined cluster approach to deliver scalable solutions for the blue economy, emphasising sustainability, innovation and systemic change. The project is supported by EcoeFISHent project 101036428/01.10.2021.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Life cycle assessment of mealworm production in closed-loop systems enhancing crop production

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Circular economy strategies and advanced agricultural technologies can reduce environmental impacts of food production; therefore, this study evaluates the life cycle assessment (LCA) of mealworm (*Tenebrio molitor*) production in refurbished containers integrated into a closed-loop system with lettuce cultivation in biopod units. The primary focus of the assessment is mealworm production, while lettuce is treated as a co-product system utilising recovered waste streams. Within this system, mealworm frass and nutrient-rich fish waster are reused as inputs for lettuce cultivation, reducing the need for external fertilisers and improving resource efficiency. Using a combination of primary and secondary data, the study assesses environmental impacts within the Biocircular project, which aims to valorise organic side streams through the integrated production of insects, fish, and vegetables. Seperate LCAs were conducted for insect and fish production to quantify their individual contributions before integrating them into a circular model that includes lettuce cultivation using recovered biofertilisers, oxygen, and carbon dioxide from insect and fish waste streams. The system boundary covers the full life cycle from raw material extraction to end-of-life, with a functional unit of 1 kg of lettuce produced per biocontainer and 27 impact categories evaluated using Environmental Footprint (EF) 3.1 methodology in SimaPro 9.6.0.1. Results show that standalone mealworm production has a climate change impact of 1.64 kgCO_2eq per kg; however, when integrated with lettuce as a co-product system, the impact is reduced to 0.948 kgCO_2eq per kg of mealworm. Additionally, lettuce production achieves a low impact of 0.0303 kgCO_2eq per kg due to the utilisation of recovered nutrients. These findings demonstrate that coupling mealworm production with lettuce cultivation in a circular system substantially improves environmental performance by converting waste streams into valuable inputs.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Milk for beetles: alternative wet feed sources for insect rearing

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In the rearing of beetle (Coleoptera) species for food and feed, the main source of feed is constituted by dry substrates, while a high-water containing substrate is used as a moisture source. Although commonly used moisture sources are agar, carrots and potatoes, these substrates pose certain limitations. Potatoes and carrots are food grade items and their use in insect rearing may not be viable from an economic standpoint, while agar is very expensive. The dairy industry stands out for producing several waste streams and byproducts that are nutritionally valuable. This trial explored these substrates as wet feed for the lesser mealworm, *Alphitobius diaperinus*. The substrates tested were expired food grade products: yogurt (Y), milk (M), kefir (K), plant-based milk (PM) and byproducts from the dairy industry: sour lactose (SoL), sweet lactose (SwL), both from yogurt production, whey (W) and sour whey (SW), both originating from ricotta production. Starch was added to each dairy byproduct (7% g/vol) to jellify it and render its administration possible to the larvae. For the control, a mixture of starch and water (7% g/vol) was used. Each treatment consisted a plastic vial containing 50 7-day-old larvae and 2 g of wheat bran. The wet feed was administered three times/week to each replicate, and larvae were sampled at weekly intervals for survival and average weight. The experiment was considered over for a specific replicate when the average weight difference between two consecutive samplings was<50%. The experimental data was analysed with RStudio software. Larval survival was always above 70%, with no significant differences among treatments ($P > 0.05$). The final weight of the larvae was also not significantly affected by the wet feed, although the highest value was obtained by the K treatment (0.018 g), higher than the control (0.014 g). The development time of the larvae was comparable for Sol, SwL, PM (42 days), K, SW, W (43.2 days) and the control (44.2 days), while longer for Y (55.3 days). Regarding bioconversion values, K performed very well, with a feed conversion ratio (FCR) value of 2.71, one of the most efficient recorded, more efficient than the control (4.03; $P < 0.05$). This treatment also allowed for one of the highest specific growth rate (SGR) values (10.58), statistically similar with the control (10.17, $P > 0.05$). This experiment explores novel sustainable substrates that could be used as wet feed sources for mass rearing protocols of *A. diaperinus*.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Using an insect for sustainable waste management of a superabundant bird

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Urban environments accumulate large volumes of faecal waste from superabundant wildlife, yet few circular strategies exist to convert this biomass into useful products. We evaluated the potential of black soldier fly (BSF; *Hermetia illucens*) larvae to bioconvert faeces from the Canada goose (*Branta canadensis*), a common bird that creates significant faecal pollution in parks and green spaces. Our objectives were to (i) test whether BSF larvae can develop on goose faeces and reduce waste, and (iii) assess whether resulting frass can serve as an effective fertiliser for duckweed (*Lemna minor*). Fresh faeces were manually collected from urban parks (Southern Quebec, Canada) using gloves, transported within 2 h, and stored at 5 °C prior to experiments. In a field survey across 11 sites, flock size strongly predicted faecal abundance (R^2 = 0.88), confirming high spatially concentrated waste inputs. In laboratory trials, 6-day-old larvae (n = 100 per replicate; 200 g substrate; three replicates per treatment) were reared on diets containing 0, 50, or 100% goose faeces, under controlled conditions (30 °C, 80% RH). Both raw and autoclaved substrates were tested to distinguish between nutritional and microbial (including pathogen-related) effects of faeces. BSF larvae successfully completed development on all diets. In raw substrates, larvae reduced dry matter by 64.0% (control), 63.5% (50% faeces), and 56.9% (100% faeces), with highest waste-reduction rates in mixed diets (3.77 g DM day^{-1}). Autoclaving reduced consumption (P = 0.007) and larval performance in faeces-only diets, indicating a beneficial role of native microbiota. Survival remained high in control (96.4%) and mixed diets (95.4%) but declined in 100% faeces (80.6%). Increasing faeces content slowed development and reduced adult body mass and lifespan (9.0 days in control vs 5.4 days in 100% faeces), linking waste quality to insect performance and biomass production. Frass derived from BSF processing was then tested as fertiliser using duckweed as a plant model. Frass significantly outperformed fresh faeces and nutrient-rich Hoagland medium: at 10 g l^{-1}, frass increased duckweed yield by 32% relative to Hoagland solution and produced higher frond numbers (6.0 vs 4.6). In contrast, fresh faeces yielded lower growth (4.7–5.0 fronds). Root length was reduced by up to 83% in frass treatments, consistent with high nutrient availability. Overall, BSF larvae efficiently converted Canada goose faeces into insect biomass and a nutrient-rich fertiliser that outperformed untreated faeces. These results demonstrate a practical circular strategy for managing wildlife-derived faecal waste in urban environments through collection, insect-based bioconversion and reuse in plant production systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Synthetic microbial community enhances the protein bioconversion from organic wastes by black soldier fly larvae

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Black soldier fly larvae are promising agents for converting organic waste into high-value biomass as sustainable alternative protein resources. However, the interactive mechanisms between the core gut microbiota and its host that govern efficient protein degradation and synthesis remain elusive. This study employed a multi-omics approach—integrating gut microbiomes, transcriptomics and metabolomics—within germ-free and gnotobiotic BSFL models to decode host-microbe interactions. Also, a defined synthetic microbial community (SynCom), rationally constructed from core taxa (*Bacillus*, *Lactobacillus*, *Issatchenkia*), was used for functional dissection. The tripartite SynCom established a stable division of labor along the gut pH gradient, maximising protein degradation and increasing final larval protein content by 63% in controlled feeding experiments. Mechanistically, the host aldehyde dehydrogenase (ALDH) was identified as a key hub, integrating microbial signals from branched-chain amino acid and tryptophan metabolism into the host's TOR pathway to boost protein synthesis. When applied to chicken manure and kitchen waste, the SynCom significantly enhanced system performance, increasing total larval protein yield (17.4 and 8.3%, respectively), feed conversion ratio (17.1 and 11.3%), and dry matter reduction (28.1 and 18.7%). Our findings provide a scalable framework for engineering insect-microbe symbiosis, enhancing the efficiency of organic waste valorisation and sustainable protein production.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Functional expression of PET-degrading enzymes in the BSF: a platform for insect-mediated plastic bioconversion

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Despite the implementation of the EU Strategy for Plastics in the Circular Economy in 2018, plastic waste management remains a major challenge, with less than 30% of the 25.8 million tonnes of plastic waste generated annually in Europe being recycled. Recycling rates increase slowly, and biological recycling approaches are limited by the scarcity of efficient enzymes and scalable systems, underscoring the need for alternative and innovative plastic valorisation strategies. This trend is particularly relevant for polyethylene terephthalate (PET), a dominant packaging plastic and major contaminant of the organic fraction of municipal solid waste, which can contain up to 5% of plastic debris. The presence of these polymers, resulting from the improper disposal of plastics, makes the management procedures of this organic waste more problematic. Black soldier fly (*Hermetia illucens*) larvae (BSFL) are widely used for organic waste bioconversion into protein-rich biomass thanks to their unparalleled feeding plasticity, but lack intrinsic plastic-degrading ability. Here, we present an innovative biotechnological platform that exploits BSFL as living bioreactors for PET biodegradation that could help address the management of plastic-contaminated waste. The approach is based on the genetic modification of BSFL to express high-performance microbial PET-degrading enzymes (PETases) in their gut. To this purpose, we selected an improved variant of a leaf-branch compost cutinase (TA-ΔLCC). Using PiggyBac-mediated transgenesis, a stable homozygous BSF line carrying a *Hi – TA – ΔLCC* construct was generated. Transgenic larvae showed the expression of LCC in the gut epithelium. Moreover, the enzyme was secreted in the gut lumen, displayed activity, and demonstrated the capability to bioconvert PET, without detectable morphological and functional alterations of the alimentary canal. Further analyses are in progress to quantify the extend of PET degradation by the larvae. These findings demonstrate the feasibility of using transgenic BSFL for integrated management of organic waste streams contaminated with PET. This approach opens new perspectives for coupling insect-based bioconversion with plastic biodegradation, contributing to circular economy strategies and offering scalable solutions for sustainable waste management.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Feeding *Tenebrio molitor* larvae (TML) with aquaponics waste for circular and sustainable production

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The DEMETRA project, "An integrated approach based on aquaponics and waste reuse for a green and smart prototype food system" (https://sostenibilita.enea.it/en/node/12802), aims to develop a sustainable food system capable of producing low-impact, high-nutritional-value food in extreme environments (e.g., underground cavities, abandoned industrial structures, large urban buildings) through aquaponics, integrating aquaculture and hydroponics. The system also incorporates waste valorisation processes, including recovery of organic matter via bioconversion (by microalgae and insects), and production of bioactive molecules and biopolymers. Within DEMETRA, *Tenebrio molitor* (TM) was selected as a model species for insect-based bioconversion. Two laboratory-scale trials were conducted. In the first, *T. molitor* larvae (TML) were reared on aquaponic dried waste (ADW) to assess its suitability as a substrate for protein production. ADW was provided as partial or total replacement of the standard diet at 50% and 100% inclusion levels, named ADW-50 and ADW-100, respectively. Growth performance was evaluated in terms of TML weight gain (as a percentage ratio between TML initial and final weight) and survival rate. In the second trial, three aquaponic crops — savoy cabbage (*Brassica oleracea* var. *sabauda*), broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis*) — were tested as wet supplements (WS) for larvae fed a standard diet. Growth performance, shelf life under rearing conditions, and larval preference (choice tests) were assessed. The best performance was observed with ADW-50, yielding a mean weight gain of 221.43%, compared to 72.41% and 79.10% for ADW-100 and the standard diet, respectively. Among WS treatments, broccoli resulted in the highest weight gain (50.19%), followed by cauliflower (38.25%) and savoy cabbage (24.66%). However, cauliflower showed higher palatability in choice tests and more consistent overall performance. These findings demonstrate the feasibility of using aquaponic by-products as feed for TML, supporting the integration of TM rearing within circular food systems. Further research will address performance under extreme conditions and the application of TML-derived products in aquafeed.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Tomato leaf waste handling affects growth performance and glycoalkaloids in desert locusts

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Tomato leaves represent a large but underutilised agricultural waste stream, yet their direct use as feed is limited by high fibre content and the presence of toxic glycoalkaloids. Feeding tomato leaves to desert locusts (*Schistocerca gregaria*) may provide a sustainable pathway for converting plant residues into high-value insect protein for food and feed applications. In this study, desert locusts were reared on tomato leaves subjected to four handling strategies: (i) freshly harvested leaves (FL), collected and fed daily; (ii) leaves stored at 8 °C for 3 days and fed daily (LS); (iii) leaves stored at 8 °C for up to 6 days with daily removal for feeding (LSD); and (iv) a leaf-cutting method (LCM), in which freshly cut stems were placed in water to maintain hydration and physiological freshness. Leaves treated with LCM remained usable for up to 7 days, thereby reducing the need for daily harvesting and feeding. Despite higher glycoalkaloid concentrations in LCM-treated leaves, accumulation of α-tomatine and dehydrotomatine in locust biomass was lower, and no significant effects on growth performance or survival were observed. The chemical composition of the produced locusts varied across treatments, with the leaf cutting method resulting in higher accumulation of Cu, Zn and Pb compared to feeding with fresh leaves. The crude protein content of the locusts remained stable across treatments (59.37–60.51%), so was their fatty acid composition. These findings suggest that the LCM strategy represents a promising approach for the development of efficient, low-labour insect bioconversion systems for agricultural waste valorisation.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Black solider fly: a potential insect for managing kitchen bio-waste

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The management of organic waste material is becoming a challenge due to the increase in global population which leads to greenhouse gas emissions and environmental loss. Our project was to evaluate the potential of larvae of black soldier fly (BSF), *Hermetia illucens* L. (Diptera: Stratiomyidae) in the management of kitchen bio-waste by evaluating their bioconversion rate and potential for sustainable waste management. Kitchen bio-waste (15 kg per house having vegetables and fruit peels and food residues) was collected from seven volunteer houses (H1-H7) for fifteen days. Waste was processed and divided into three 5 ± 0.1 kg replicates per household. The H1 and H4 kitchen bio-waste gave the highest larval growth compared to H2, H3, H5, H6 and H7, showing that waste composition affects larval development. Moreover, in comparison to the waste residues from the other houses, H1, H2, H3, H4, H6 and H7, the kitchen bio-waste residues from house H5 showed the highest weight. This suggests that even though each bin contained 600 first-instar larvae and was divided into three equal pieces, the quality or content of the 15 kg of kitchen bio-waste collected in H5 did not favour larval development more than that of the wastes in H1, H2, H3, H4, H6 and H7. Black soldier fly larvae expressed as an effective waste manager, which is also cost-effective and sustainable method of waste management, with also production of useful products of protein-enriched larvae biomass (used in animal feed) and frass (used as fertiliser for plants).

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Dairy sludge as a rearing diet for black soldier fly larvae (*Hermetia illucens* L.): Successful? Efficient?

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Black soldier fly larvae (BSFL) are known for their capacity to valorise diverse residual streams by converting them into valuable resources. This study evaluated BSFL rearing efficiency on industrial dairy sludge (DS), alone or mixed with different materials to improve the sludge's nutritional and physical properties. BSFL were reared for 7 days on wheat bran, dairy sludge, DS mixed with wheat bran (80:20 weight-based), and DS mixed with sawdust (80:20 weight-based), and for 14 days on DS alone (DS-14). For each diet, four crates (replicates) were prepared, each with 10 kg fresh diet and 18 500 5-day-old BSFL. Dietary crude protein ranged from 18.1% in wheat bran to 46.6% in DS, while crude fat ranged from 1.6% DS-sawdust mix to 4.9% in the DS-wheat bran mix. Assessed parameters included larval growth (harvested total mass, individual weight, growth rate and survival), waste reduction index (WRI), efficiency of conversion of ingested feed (ECI), and biochemical composition of larvae and frass. Larvae reared on DS for 7 days had the highest harvested total mass (1,720 g), individual weight (63.6 mg), growth rate (8.3 mg per larva/day), and ECI (0.3). DS-14 larvae had the highest WRI (11.7 g/day) but the lowest ECI (0.1). Wheat bran-fed larvae showed poor growth, with the lowest harvested total mass (1,073 g) and growth rate (6.1 mg per larva/day). DS mixed with sawdust resulted in the lowest WRI. Larval crude protein ranged from 42.2–48.3% (DM basis), and did not directly reflect dietary protein. Larval crude fat was highest on wheat bran (9.0% DM basis) and lowest (2.9% DM basis) on DS-14. Frass from DS-7 had the highest nitrogen (6.8% DM basis) and phosphorus (4.6% DM basis) contents, while frass from DS mixed with sawdust had the lowest (2.2% and 1.3% DM basis, respectively). In conclusion, these findings indicate that DS can solely support BSFL rearing under the tested conditions, and adding 20% wheat bran or sawdust did not improve performance. This highlights the potential of dairy sludge as a sustainable substrate for insect-based bioconversion, contributing to circular economy strategies and sustainable nutrient recycling in agro-industrial systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Efficacy of thermophilic aerobic fermentation of pig carcasses and subsequent bioconversion by black soldier fly larvae

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The management of animal carcasses remains a critical environmental and logistical challenge for livestock production systems. Conventional disposal methods present major limitations: burial poses long-term risks to groundwater contamination; composting is time-consuming and associated with substantial greenhouse gas emissions; incineration and rendering are highly energy-intensive; and, except for rendering, most approaches result in a near-complete loss of recoverable nutrients. Solid-state aerobic digestion represents a potential alternative, in which thermophilic bacteria drive rapid microbial activity and heat generation. We hypothesize that a thermophilic fermentation of 24 h may allow biological stabilization by inactivating pathogenic micro-organisms, while producing a microbiologically stable digestate rich in nutrients for subsequent valorisation. In this study, two fermentation approaches of pig carcasses were tested using *Bacillus smithii* as the thermophilic bacterium, at pork carcass:sawdust ratios of 2:1 and 3:1 (w/w). A standard Gainesville diet and a non-fermented 3:1 pork carcass:sawdust mixture were used as controls, and a 50:50 (w/w) mixture of Gainesville diet and fermented 3:1 substrate was also evaluated. Five-day-old black soldier fly larvae (BSFL) were reared on each substrate for 7 days (500 larvae per treatment, 0.2 g DM/larva, three replicates). Bioconversion efficiency differed significantly among diets (one-way ANOVA, $p < 0.0001$). The fermented 3:1 pork carcass:sawdust substrate outperformed its non-fermented counterpart by 26.3%, while supplementation of the fermented 3:1 substrate with the Gainesville diet at a 50:50 ratio resulted in a 30.8% higher bioconversion efficiency compared to the Gainesville diet alone. In contrast, the fermented 2:1 treatment showed significantly lower performance. Overall, thermophilic aerobic fermentation has the potential to biologically stabilize carcass waste while producing nutrient-rich digestates that support efficient BSFL bioconversion, both as a substrate and as a supplementation.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

When apple meets olive: nutrient balanced pomace diets driving black soldier fly density and performance

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Agricultural processing generates large volumes of byproducts every year, and in Mediterranean regions, olive and apple pomaces account for a large portion of these residues. Finding effective ways to repurpose these materials remains a challenge, making the search for alternative valorisation pathways increasingly relevant. Black soldier fly larvae (BSFL) are known for their ability to consume a wide range of organic byproducts and are considered efficient bioconverters. This study evaluated the impact of isonitrogenous and isoenergetic diets on larval density and overall performance. Three diets were formulated using apple pomace, olive pomace, or a combination of both, each supplemented with wheat bran and brewer's spent grains. These diets were tested at three larval densities: 1, 2.5 and 5 larvae/cm^2. Larval weight and survival were recorded every three days, and the experiment ended once 10% of the larvae reached the prepupal stage. Larval protein content was also analysed. The results revealed significant differences across all measured parameters except survival, which remained higher than 90% for all treatments. Individual larval weight varied among treatments, with the olive pomace diet at the highest density producing the heaviest larvae (96 mg), followed by the apple pomace diets across all densities (82-90 mg). The lowest weights were recorded in the olive pomace diet at the lowest density (60 mg). Development time also differed, with the apple pomace diet at the highest density reaching prepupal stage in just 6 days, while all olive pomace diets required around 12 days. Larval crude protein ranged from 48 to 56%, with the highest value recorded in the apple pomace diet at 5 larvae/cm^2 density and the lowest in the olive pomace diet at the highest larval density. Collectively, these findings highlight the strong influence of substrate type and larval density on BSFL growth and efficiency. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

From Mediterranean waste to insect protein: larval performance of *Hermetia illucens* on two-phase olive Pomace diets

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Olive pomace is one of the most abundant byproducts in Mediterranean regions, generated during olive oil extraction. The valorisation of two phase olive pomace (TPOP) remains challenging, mainly because of its high moisture content (>60%). The black soldier fly (BSF) is widely recognized as an efficient bioconverter capable of utilising a broad range of organic byproducts. Considering the species' preference for moist substrates, this study examined the partial inclusion of TPOP in BSF larval diets alongside chicken feed. Both dried TPOP (74 °C for 5 h) and wet TPOP were tested at inclusion levels of 10, 15, 25, 50 and 75% for the dried form, and 5, 10, 15, 25, 50 and 75% for the wet form. A 100% chicken feed diet served as the control, and all diets were adjusted to 70% moisture. Larval survival and weight were recorded every three days until 10% of the larvae reached the prepupal stage, marking the end of the trial. Protein and lipid content were subsequently analysed, and feed conversion ratio (FCR) and bioconversion rate (BCR) were calculated. Significant differences were observed across nearly all measured parameters except survival, which remained above 94% in all treatments. The highest larval weights were recorded in the control diet (146.4 mg), with no significant differences compared to both TPOP forms up to the 25% inclusion level. Diets containing more than 50% TPOP resulted in reduced individual larval weights. The 25% wet TPOP diet showed the best FCR and BCR values (7.6 and 13.2%, respectively), whereas the 75% dried TPOP diet led to the poorest performance and the highest crude lipid content (around 34%). Protein content increased with higher inclusion levels of dried TPOP, reaching 47% at the 50% level, but dropped to 39.6% at 75% dried TPOP diet. Taken together, these results demonstrate the potential of incorporating TPOP into BSF larval diets and emphasize how drying and inclusion level shape both nutritional outcomes and larval growth performance. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Safety assessment of black soldier fly larvae reared on non-permitted substrates

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Black soldier fly larvae (BSFL; *Hermetia illucens*) can convert diverse organic residual streams into valuable biomass, including materials currently excluded under EU feed legislation, which offers opportunities for circularity. Within the Safe Insects 1 project, we assessed BSFL performance and food safety outcomes on several non-authorised substrates.

Feeding trials used a control diet, kitchen waste, supermarket waste, chicken manure and category 2 meat meal. Pre studies showed that category 2 meat meal was unsuitable for BSFL and was excluded. All substrates and larvae were analysed for chemical contaminants, heavy metals, pesticides, PFAS, dioxins, PCBs, veterinary drugs and microbiological hazards. To study pathogen behaviour, chicken manure was spiked with *Salmonella enterica*, *Clostridium perfringens*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Eimeria oocysts* and five viruses. A quantitative risk assessment evaluated the likelihood of prion transfer.

Across all substrates, dioxins, PCBs and PFAS were detected only at low levels. Kitchen waste and supermarket waste had high microbial loads, including C. perfringens and *B. cereus*, while *Salmonella* was found only in supermarket waste. Kitchen waste and supermarket waste contained pesticides, and supermarket waste also showed unexpected high nicotine levels. Heavy metals were present in both substrates. Cadmium accumulated in BSFL reared on kitchen waste but remained below legal limits. Feeding on supermarket waste resulted in bioaccumulation of several elements, although BSFL concentrations stayed within regulatory thresholds. Chicken manure contained elevated metals and coccidiostats, which did not accumulate in BSFL but exceeded carry over limits in the substrate.

Some pathogens transferred from the spiked substrate to the BSFL, while others were not detected. Two viruses appeared only on day 1, whereas hepatitis E virus and *Eimeria* persisted in the BSFL. *B. cereus*, *K. pneumoniae* and *S. abaetutuba* were found in both substrate and BSFL, though pre-existing bacteria complicate interpretation. *C. perfringens* appeared only in the substrate. Lasty, the quantitative risk assessment concluded that prion transfer from insects reared on these substrates is very unlikely. A follow up project, Safe Insects 2.0, is examining animal by product residual streams, focusing on processing effects on the presence of animal DNA and microbiological risks, and the legal conditions for safely using currently unauthorised substrates.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Progress on research and application of black soldier fly and microbes in China

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Over the past twenty years, investigators in China have made great progress on basic research and industry application of black soldier fly (BSF) and microbes. We bred Chinese local black soldier fly Wuhan strain, sequencing its genome and discovered a large amount of active substances in black soldier flies, such as antimicrobial peptides and active enzymes. We first found that the gut microbiota is an essential organ of the black soldier fly. Subsequent studies have found that key microorganisms in the gut of black soldier flies play different roles in the conversion of organic waste, such as promoting protein degradation in organic solid waste by black soldier flies, promoting egg laying by adult black soldier flies, degrading cellulose in materials, promoting the growth and development of black soldier flies, assisting black soldier fly larvae in inhibiting zoonotic pathogens in substrates (such as livestock manure), degrading antibiotic residues in livestock manure and reducing antibiotic resistance genes and their mechanism. We are on the way to explore exactly low cost and high efficiency conversion process and equipment of organic solid waste, and plays an important role in alleviating the shortage of soybean and fish meal in China. We have established safe conversion and detection technologies, and the conversion products of black soldier flies have been included in the Chinese feed catalogue. We have also discovered some BSF pathogens and find the green control methods, and established national, group and local standards in BSF breeding, conversion process and its production (BSF larvae powder) for black soldier flies industrialization.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Olive pomace and goat manure valorisation by anaerobic digestion and bioconversion with *Hermetia illucens*

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The increasing demand for sustainable management of agro-industrial residues has intensified interest in circular bioeconomy strategies integrating energy recovery and material valorisation. Olive pomace (OP) and goat manure (GM) are abundant organic wastes with high potential for biological conversion, although OP recalcitrance often limits bioconversion efficiency. This study investigated an integrated two-stage pathway combining anaerobic digestion (AD) and bioconversion using *Hermetia illucens* (black soldier fly larvae, BSFL). In the first stage, the effect of OP thermal pre-treatments (90, 120 and 150 °C) on biogas production performance was assessed. Pre-treated OP-GM (2:1, dry weight) were anaerobically digested under mesophilic conditions (37 °C, 17 days) and compared with an untreated OP-GM and GM. In the second stage, solid digestates, untreated OP-GM, raw OP and GM were used as rearing substrates for BSFL to compare performance on digested and non-digested substrates. Survival, growth and bioconversion efficiency, using a standard chicken feed diet as control. Phytotoxicity of digestates obtained from anaerobic digestion and frass produced by larvae reared on a raw OP-GM was evaluated using a germination index (GI) on alfalfa seeds. The 120 °C pre-treatment yielded the highest cumulative biogas pressure (1400 hPa), whereas GM reached about 500 hPa. However, untreated OP-GM showed comparable yields, indicating limited benefits of thermal pre-treatment. BSFL reared on digestates exhibited high survival rates (up to 98%) but reduced growth, with final larval weights of 0.09–0.11 g on digestates, compared to 0.083 g on untreated OP-GM, 0.08 g on standard diet, 0.03 g on raw OP and 0.04 g on GM. Bioconversion efficiency decreased from 18.7% (standard diet) to below 12% on digestates. Phytotoxicity tests revealed strong inhibitory effects for digestates (GI 7–10%), whereas frass showed no phytotoxicity, with GI exceeding 130%. Overall, thermal pre-treatment does not significantly improve AD performance and may compromise digestate suitability for insect bioconversion. Conversely, the AD-BSFL approach without thermal pre-treatment represents a promising strategy for renewable energy recovery, waste reduction and the production of stable, agronomically valuable frass and insect protein.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

BioInsectonomy: a trinational collaborative project for circular insect-based feed production from agricultural residues

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BioInsectonomy is a trinational collaborative research project involving institutions from Germany, Colombia, and Norway, aimed at advancing circular solutions based on insect farming for sustainable feed production. The project was developed by integrating complementary expertise while conducting experiments in different regions. The European members addressed analytical validation by developing four real-time PCR assays for the species-specific identification of insects in feed, as well as mass spectrometry-based methods for the detection of insect and non-insect peptide markers. In Colombia, researchers analysed the production chain from the use of agricultural residues as substrates for insect, larval rearing, processing, to the formulation of insect meals, evaluating the performance of four diets in two insect species, *Hermetia illucens* and *Tenebrio molitor*. Insect meals were produced using harmonized processing protocols and analysed to assess their safety. Feeding trials conducted in Colombia evaluated four aquaculture diets formulated with *H. illucens* meal in the tropical fish *Piaractus brachypomus*, indicating that this insect-based ingredient could be incorporated into aquaculture feeds without adverse effects under the experimental conditions. In parallel, guidelines and standard operating procedures for the establishment and operation of insect farms producing processed animal proteins were jointly developed in accordance with both Colombian and EU regulatory frameworks. These activities were embedded in a shared vision to strengthen long-term scientific and technical cooperation between the EU and Colombia. More than 8 scientific papers and a review authored by all unit heads were drafted for international peer-reviewed journals. Overall, the results demonstrate the feasibility of integrating insect production, analytical control, and regulatory alignment within a coordinated international research framework, illustrating how sustained trinational collaboration can support the development of safe, traceable and context-adapted insect-based feed systems aligned with bioeconomy and sustainability strategies.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Black soldier fly frass as a multifunctional input for sustainable crop protection and productivity

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Agricultural waste management represents a major environmental and economic challenge worldwide. The use of black soldier fly larvae (*Hermetia illucens*) for bioconversion of agricultural residues into insect biomass is increasingly recognized as a key component of circular agri-food systems. This process generates frass as a secondary product composed of larval excreta, exoskeleton residues, and partially digested organic matter. Despite its increasing availability, the biological and agronomic properties of BSF frass remain insufficiently characterised. In this study, we evaluated BSF frass as a multifunctional input for sustainable crop production, focusing on its effects on plant performance, pest and disease incidence, and soil microbial communities. Frass samples derived from different production systems underwent thermal stabilization prior to application. Chemical properties were characterised, and laboratory and field experiments were conducted using tomato and cucumber as model crops. Frass application consistently enhanced plant vigor and yield compared to untreated controls and was associated with reduced pest and disease incidence. Analysis of soil bacterial communities revealed that frass treatments did not alter overall taxonomic richness or evenness but significantly reshaped community composition and assembly patterns. Incorporation of frass as a treatment variable contributed to explaining between-group differences in microbial profiles, primarily through shifts in the relative abundance of low-abundance but agriculturally relevant bacterial groups. Notably, frass-amended soils tended to be enriched in nitrogen-fixing bacterial groups and depleted in groups associated with reduced nutrient availability. Overall, frass inputs favoured specific functional groups without causing broad losses in bacterial diversity. Our results demonstrate that BSF frass functions not only as a nutrient source but also as a biologically active input capable of shaping soil microbial communities in ways relevant to crop productivity. These findings highlight the potential of BSF frass as a value-added product within insect-based waste management systems and support the need for classification and standardization of frass products based on both chemical and microbial characteristics to enable reliable and scalable application in sustainable agriculture.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Advances in the production and application of insect frass fertilisers

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Insect frass fertiliser is gaining global recognition as high-value product for enhancing soil health and crop productivity. There is increased diversification of frass fertiliser in addressing soil health and crop production challenges. This study presents recent efforts in frass fertiliser optimization and application for soil fertility management, yield enhancement and pest management. We evaluated the effect of pit and heap composting and influence of phosphate rock (PR) amendments (5 – 25%) on the quality, yield and profitability of black soldier fly frass fertiliser (BSFFF) production. We used agronomic studies to assess of different BSFFF products (solid, liquid, chitin-fortified solid, chitin-fortified liquid on broccoli yield and nutritional profiles in comparison with conventional organic fertiliser (Safi) and unfertilised soil (control). Lastly, we assessed the pest control effects of insect oil/neem oil-BSFFF biorationals against tomato leaf miner and fall armyworm using application rates of (25, 50 and 100 µl/ml). Findings showed that composting methods did not significantly affect nutrient levels, but the heap method produced BSFFF with high-level of maturity, indicated by lower carbon to nitrogen ratio (< 12) and higher germination index (> 80%). Heap composting also yielded 7% more frass and higher net income. We noted that amendment of substrates with PR yielded 1.1 – 1.4-fold more BSFFF. The BSFFF with 20% PR inclusion had 2.9 – 7.8-fold and 1.1 – 1.7-fold higher concentrations of phosphorus and macronutrients, respectively, compared to unamended fertilizer. Field studies showed that soils amended with BSFFF products produced broccoli with 26 – 138% more yield and 4.6-fold higher agronomic use efficiency compared to Safi and control treatments. Liquid BSFFF produced broccoli with higher levels of crude fat (61%), carbohydrates (16%), and calcium (38%) compared to other BSFFF products while the highest crude protein, potassium, crush ash and phosphorus were achieved using chitin-fortified BSFFF. Bioassays showed no significant differences in pest mortality between insect oil- and neem-oil-based biorationals. Pest mortalities of 34 – 93% and 11 – 94% were achieved for tomato leaf miner and fall armyworm, respectively, with efficacy increasing at higher concentrations. Our findings provide insights for optimising frass fertiliser production and diversification to address biotic and abiotic challenges to crop production, thus contributing to improved soil health, food security and climate resilience through circular economy.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Research on the efficiency and application prospects of black soldier fly cascading degradation of high-fibre waste

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With global population growth and changes in consumption patterns, the production of high-fibre organic waste (such as agricultural straw, distiller's grains, and fruit and vegetable residues) continues to rise. Due to their high cellulose, hemicellulose, and lignin content, these wastes are difficult to efficiently degrade using conventional biological methods, making their treatment a major challenge in the field of solid waste management. The development of efficient and environmentally friendly resource utilisation technologies is urgently needed. Black soldier fly larvae, as resource insects, have shown great potential in organic waste treatment. Current research has mainly focused on their ability to process food waste, livestock manure, and other similar materials, while systematic studies on their degradation of high-fibre waste remain insufficient. This study aims to thoroughly investigate the degradation efficiency of black soldier fly larvae on high-fibre waste and the feasibility of large-scale application. This study evaluated the growth and waste reduction effects (weight reduction rate, fibre component degradation rate) of black soldier fly larvae and cascading transformation processes on high-fibre substrates primarily composed of distiller's grains and corn straw. The results showed that using black soldier fly larvae (which are more adaptable to high-moisture substrates) to decompose organic matter in fresh distiller's grains, followed by the introduction of yellow mealworm larvae (which have stronger cellulose decomposition capabilities) for subsequent degradation, proved effective. This insect cascading biotransformation technology can efficiently convert distiller's grains into insect biomass rich in protein and balanced in amino acid composition, providing high-quality raw materials to address the shortage of feed protein in China. This solution not only effectively tackles the challenges of treating high-fibre waste such as distiller's grains but also contributes to promoting the sustainable development of agricultural production and ecosystems. Future research should focus on optimising pretreatment processes, screening efficient synergistic degradation strains, and promoting the engineering demonstration and application of this technology.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Enhancing the nutritional value of olive pomace via microbial fermentation for *Hermetia illucens* larvae production

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The escalating global demand for protein-rich feed necessitates the urgent development of sustainable alternatives. Black soldier fly larvae (BSFL) represent a promising solution due to their high nutritional value, including proteins, minerals, and beneficial fatty acids. BSFL can be reared on agricultural by-products such as olive pomace (OP), a by-product of olive oil production that poses an environmental challenge due to its large volumes and high toxicity. This study evaluated the potential of olive pomace as feedstock for BSFL rearing following different biological pretreatments. Experiments were conducted by inoculating OP with bacterial strains (*Bacillus subtilis*, *B. pumilus*, and *B. megaterium*) or fungal strains (*Trametes versicolour*, *Pleurotus eryngii* and *Phanerochaete chrysosporium*) under controlled temperature and moisture conditions. The effects of inoculum proportion, medium composition, and substrate sterilisation were assessed under both solid-state fermentation (SSF) and submerged fermentation (SF) conditions. The efficacy of the biologically pretreated OP was assessed through laboratory-scale BSFL growth trials by evaluating survival and larvae biomass and compared to a control feed (chicken feed) and non-treated OP. Larvae grow on non-treated OP for nine days showed a biomass that was 20% of the biomass obtained in the control feed (chicken feed). Biological treatment of OP with *T. versicolour* under SSF conditions significantly increased BSFL biomass to 40–60% of the control biomass. In contrast, bacterial pretreatment with *B. pumilus* and *B. subtilis* under SF conditions improved BSFL growth, achieving approx. 80% of the biomass observed in the control. The highest BSFL biomass was obtained when OP was mixed with wheat bran (WB) and pre-digested with *T. versicolour*, *P. eryngii* or *P. chrysosporium* under SSF, or with *B. pumilus* and *B. megaterium* under SF conditions, exceeding the control biomass by 20–40%. In conclusion, fungal pretreatment of OP under solid-state fermentation alone showed limited effectiveness in improving its digestibility for BSFL. Submerged fermentation with bacterial strains yielded more promising results but requires further optimisation. The best overall performance was achieved through co-digestion of OP with wheat bran under both SSF and SF conditions, confirming co-digestion as one of the most effective strategies for BSFL rearing.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Fish side-streams to high-value resources: environmental and economic sustainability of black soldier fly biorefinery

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The transition towards a circular Blue Economy requires internalizing industrial residues through "zero-waste" systems. In this framework, the EcoeFISHent project utilises the black soldier fly (BSF), which consumes fish side-streams, as a biological engine to upcycle them into high-demand resources: chitin, oil, and organic fertilisers. Despite growing interest, integrated environmental and economic assessments of multi-output insect production systems remain limited, especially at industrial scale. This study presents a combined life cycle assessment (LCA) and life cycle costing (LCC) of an industrial BSF process rearing on fish side-streams to co-produce oil, chitin, and frass fertiliser. A "cradle-to-gate" approach was applied, covering the entire value chain from feed preparation — fish side-streams (approx. 20% mass) and agricultural by-products (wheat and rice bran) — to the extraction of final co-products. Primary data from the Nasekomo facility demonstrate high bioconversion efficiency, with biomass scaling from 959 g of 5-DOLS larvae to 39 235 g of 12-DOLS larvae, and will be corroborated on the EcoeFISHent larva bioconversion pilot facility in UNITO and integrated with secondary data from reference databases. Results indicate environmental impacts consistent with, and in some categories lower than, literature benchmarks. Global Warming Potential (GWP) was 26.6 kg CO_2 eq/kg chitin and 27.8 kg CO_2 eq/kg oil, representing a 75–97% reduction compared to conventional crustacean systems due to avoided chemical demineralization. The protein-rich pulp is used for oil extraction, while the remaining fraction is reused as larvae feed, enhancing circularity. Electricity is the dominant contributor (approx. 95% of GWP and Fossil Resource Depletion), mainly in extraction, thermal deactivation, and deskinning. Wheat bran drives approx. 97% of Land Use and Freshwater Eutrophication due to upstream agricultural burdens. The valorisation of BSF frass plays a key role in nutrient loop closure and significantly improves economic performance. Economic modelling estimates production costs of approximately €2.80–5.20 per kg of output, aligning with current market price ranges. Its value lies in returning organic matter and nutrients to soil and displacing synthetic fertilisers associated with high environmental burdens. Overall, this study demonstrates the environmental and economic viability of BSF bioconversion as a zero-waste solution for the fishing sector. Future efforts should prioritize renewable energy integration and replacing agricultural substrates with fishery side-streams to further enhance sustainability.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Olive it or leave it: valorising olive fragments in black soldier fly larvae diets

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Olive oil production generates a wide range of by-products, including olive fragments, mainly in Mediterranean countries. These fragments, mostly olive leaves and branches, are often discarded after harvesting, although they retain nutritional potential. Black soldier fly larvae (BSFL) can efficiently utilise such agricultural residues and convert them into high-quality protein. Therefore, this study investigated the impact of incorporating different levels of olive fragments into BSFL diets on larval growth and performance. Diets with three inclusion levels of olive fragments (5, 12 and 25%) were combined with whey, brewers' spent grains and wheat bran, while the control diet contained the same by-products without olive fragments. All diets were formulated to meet the larvae's protein and lipid requirements, except for the 25% olive fragments diet, which had a slightly lower protein content; three of the diets were isoenergetic. Larval performance was evaluated every three days by recording weight and survival until 10% of the larvae reached the prepupal stage. Feed utilisation parameters, specifically Feed Conversion Ratio (FCR) and Bioconversion Ratio (BCR), were calculated, and nutritional analyses of diets and larvae were performed. Survival remained higher than 90% for all diets, with no significant differences. The control diet and the 5% inclusion diet consistently showed the best performance across all the evaluated parameters. The 12% olive fragments diet demonstrated intermediate results, while the 25% diet performed the least favourably. Chemical analyses revealed that larvae contained 50–56% protein and 2–9% lipids, with the highest values being recorded for the 5% and control diet, respectively. In conclusion, these findings indicate that moderate inclusion of olive fragments in BSFL diets can enhance feed efficiency and growth, whereas higher levels may reduce performance. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA-Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging, and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Closing the loop: evaluation of tomato by-products as feed for *Tenebrio molitor* larvae

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During industrial tomato processing, large amounts of by-products are generated and often remain underutilised. Within a circular economy framework, these by-products could be used as alternative feeding substrates for *Tenebrio molitor* larvae, contributing to sustainable insect production. The aim of the present study was to evaluate the suitability of tomato by-products, mixed at different ratios with wheat bran, as diets for *T. molitor* larvae. The diets were formulated with tomato by-products at inclusion levels of 10, 25 and 50%, while two controls were used: 100% wheat bran and 100% tomato by-product. Larval performance was evaluated on a weekly basis by recording larval weight and survival, and the experimental period ended with the appearance of the first pupa. In addition, feed utilisation parameters, such as Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR), were calculated. Analyses for proteins and lipids were performed in larvae and diets, while tomato by-products were tested for lycopene. Statistical analysis revealed significant differences among most evaluated parameters, while survival rate remained above 83% in all cases. Wheat bran showed the highest overall performance, followed by tomato inclusion levels of 10% and 25%. The 50% tomato diet presented intermediate to lower performance, whereas the 100% tomato diet was less efficient across all parameters. Regarding larval chemical composition, protein content was approximately 52% across most diets, whereas larvae fed exclusively on tomato by-products showed notably higher results (approx. 69%). Lipid content was around 30% in larvae from all diets, but was markedly reduced (approx. 3%) in the 100% tomato treatment. The analyses of tomato by-products indicated the presence of lycopene that may act as a beneficial factor for *T. molitor* larvae. These findings suggest that tomato by-products can be effectively used when mixed with other substrates, contributing to the optimisation of *T. molitor* larval production.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Mitigating antimicrobial resistance transmission in manure via black soldier fly larvae-microbe synergistic conversion

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The dissemination of antibiotic resistance genes (ARGs) is a critical factor driving the global increase in environmental antimicrobial resistance (AMR), with animal manure being a primary reservoir posing significant public health risks. Black soldier fly larvae (BSFL) treatment is an emerging agricultural waste valorisation technology that simultaneously addresses pollution and produces insect biomass for feed protein. This study focuses on controlling the environmental risks of ARGs during BSFL conversion and elucidating the underlying microbial mechanisms. Our results demonstrate that BSFL-microbe synergistic conversion effectively reduced the total absolute abundance of ARGs in pig manure by 93.2%, significantly mitigating the risk of multidrug-resistant pathogens. Field experiments using insect frass as biofertiliser revealed a specific microecological mechanism: the application of frass activated the Bacillaceae family in the soil. This bacterial family, known for inhibiting antibiotic-resistant bacteria (ARB), showed enhanced carbohydrate metabolism pathways and promoted biofilm formation, which antagonized ARB and consequently reduced ARG proliferation. Furthermore, tracking experiments in radish (*Raphanus sativus*) cultivation showed that frass application significantly decreased the richness and abundance of ARGs, mobile genetic elements (MGEs), and virulence factors (VFs) in the edible roots compared to conventional fertilisation, thereby reducing potential pathogenic resistant bacteria. This study provides comprehensive technical and theoretical support for mitigating the transmission of antibiotic resistance in agricultural environments, highlighting the safety and ecological benefits of BSFL-derived biofertilisers.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Integrating dairy side-streams into insect rearing: performance of *Hermetia illucens* and *Tenebrio molitor* larvae

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Dairy side-streams are generated in large volumes and may pose environmental risks due to their high organic load, odours and microbiological hazards when inadequately managed. Although these materials are often redirected to biogas production, insect bioconversion provides an additional circular pathway for nutrient recovery. Here, we evaluated expired dairy products and side-streams from cheese production as rearing inputs for two commercially relevant insect species: the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), and the yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Across all dairy-based treatments, *H. illucens* larvae showed rapid development (10–13 days) and high survival (>90%), with expired yogurt consistently yielding the best performance, including higher larval weight and improved feed conversion ratio. For *T. molitor*, dairy side-streams were provided as moisture sources with agar or starch as gelling agents. All supported successful development, with high final larval weights and survival generally >80%, while development time was shortened (<49 days) in some treatments. Proximate larval composition analyses indicated no treatment effects on larval nitrogen content in either species, but this was not true in the case of lipids. This research is supported by PRIMA, a program supported by the European Union, under grant agreement No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

From soil to water: agronomic performance and environmental safety of black soldier fly frass as a sustainable fertiliser

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The transition towards sustainable fertilisation strategies requires alternatives to synthetic mineral fertilisers that maintain crop productivity while reducing environmental impacts. Insect frass derived from *Hermetia illucens* larvae represents a circular, bio-based fertiliser with potential to improve soil functioning and nutrient retention. However, integrated assessments across soil–plant systems and aquatic compartments remain limited. Two entomofertilisers produced from *H. illucens* frass (olive pomace-based and cereal-based) were evaluated against a conventional mineral NPK fertiliser using integrated experimental systems. Soil–plant assays with *Brassica rapa* were conducted over two full life cycles under equivalent nitrogen inputs to assess plant performance, soil enzymatic activity, nutrient dynamics, and soil ecotoxicology. In parallel, soil column experiments simulated intense rainfall to quantify nutrient leaching (NH_4^+, NO_3^-, PO_4^{3-}), and leachate ecotoxicity to freshwater organisms was assessed. Frass-based fertilisation supported plant development comparably to 100% NPK, particularly when combined with mineral fertiliser, while exclusive frass applications showed slightly lower early-stage biomass, consistent with the slow-release dynamics of frass. Plant physiological indicators remained stable, indicating no metabolic stress. Frass amendments enhanced soil enzymatic activities related to C, N, and P cycling and promoted balanced ammonium–nitrate dynamics. Soils amended with frass sustained plant growth in a second cultivation cycle without re-fertilisation, demonstrating residual fertility. Leaching experiments showed lower N and P losses from frass-amended soils than from NPK-treated soils. Soil ecotoxicological assays revealed no adverse effects on soil invertebrates, and frass leachates induced no toxicity in aquatic organisms, whereas NPK leachates inhibited algal growth. *H. illucens* frass is an environmentally safe and effective bio-based fertiliser that supports plant performance, improves soil health, reduces nutrient losses, and limits ecological risks, making it a viable alternative or complement to synthetic fertilisers in circular and climate-smart agriculture.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Colonisation of the black soldier fly larval midgut by *Escherichia coli* for improved biotransformation strategies

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Insect-mediated bioconversion represents a promising approach for the valorisation of organic wastes and by-products of the agri-food chain, enabling the production of high-value insect biomass. Black soldier fly larvae (BSFL) are among the most effective bioconversion agents, industrially exploited to obtain protein for animal feed, and extensively studied for a wide range of biotechnological applications, including sustainable waste management strategies. Bioconversion of organic waste may be challenging due to the presence of recalcitrant, hardly digestible, polymers, such as plastics or lignocellulose. Their breakdown during the bioconversion process could be facilitated by conditioning BSFL midgut microbiota with engineered bacteria producing specific hydrolytic enzymes and making available degradation products to boost BSFL growth. To this purpose, protein engineering and synthetic biology made *Escherichia coli* a valuable biotechnological tool for the expression of single or multiple genes of interest. However, *E. coli* is not a natural inhabitant of BSFL gut. For this reason, it is important to assess whether this bacterium can colonize the BSFL gut. In the present work we developed an effective and simple protocol to monitor the colonization dynamics of BSFL midgut by *E. coli*. In particular, we: (i) determined the factors affecting *E. coli* survival in different BSFL rearing substrates and in the larval midgut; (ii) identified simple, cost-effective strategies to improve the persistence of *E. coli* in the BSFL midgut; and (iii) verified the expression of a newly introduced gene in midgut-colonizing *E. coli* by RT-qPCR. The results showed that the engineered *E. coli* can colonise the BSFL midgut and proved to be metabolically active. However, the outcome of the colonisation largely depends on the rearing substrate used. In particular, evidence suggested an antagonistic role of substrate and midgut microbiota, both driven by chemical composition of the diet, against *E. coli*. Importantly, the metabolically active bacterium can produce the protein of interest after several days post-inoculation in the substrate. Overall, this study demonstrated that recombinant *E. coli* can colonize BSFL midgut and could be used as a tool for engineering midgut microbiota to support larvae digestive functions.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Feeding *Alphitobius diaperinus* larvae with adults of *Aedes albopictus* and *Bactrocera dorsalis*

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Insects are mass-reared for pest-control programs (e.g., Sterile Insect Technique (SIT)) and for food and feed applications. A common yet underutilised byproduct of these systems are the parental adults that remain after mating, having no further operational value. Developing valorisation pathways for this "adult" side-stream could improve the sustainability and profitability of mass-rearing facilities. One promising approach is their conversion through secondary insect production systems, such as the one of the lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), which can efficiently convert organic substrates into high-nutritional biomass and more. In this study, we assessed adult side-streams from the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae) from a mass rearing lab, and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), as dietary ingredients for *A. diaperinus* larvae. Wheat bran-based diets were formulated with 0, 25, 50, 75, or 100% (w/w) adult side-stream from each species. The results indicated that inclusion of 25 to 75% adults of both species in the *A. diaperinus* larval diet improved multiple developmental traits in comparison to the wheat-bran control treatments, whereas performance at 100% inclusion was less favourable. These findings support the concept that adult Diptera can function as a valuable feed ingredient for *A. diaperinus* production and highlight an opportunity for integrated, circular use of this biomass across insect-rearing facilities. Further work should characterise the nutritional drivers of the positive responses observed, and particularly which nutrients are complemented by the dipteran adults that wheat bran is lacking, in order to refine *A. diaperinus* diet formulations. Acknowledgement: This research is supported by PRIMA, a program supported by the European Union, under grant agreement No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Agro-industrial by-products for black soldier fly larvae: gut microbiota, performance and probiotic application

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Larvae of black soldier fly (*Hermetia illucens* L.) efficiently bioconvert low value organic mass into high quality biomass enabling the valorisation of agri-food side-streams within circular bioeconomy paradigms. Scaling up black soldier fly production in a sustainable and cost efficient manner, requires the identification of feedstocks economically accessible and available at industrial volumes. Diet composition is known to modulate not also larval performance, but also gut microbiota, a key determinant of host physiology including nutrition and development. Larvae were reared on agri food by products (okara, brewer's spent grain, potato peels, and potato sorting residues) alongside a standard diet. Growth performance was quantified, and gut microbial communities were profiled in terms of taxonomic composition and total bacterial load. Moreover, to enhance larval performance on potato wastes, four bacterial strains were administered via the substrate with two inoculations; heat inactivated counterparts were also provided to evaluate their contribution as a source of nutrients. Okara supported the highest bioconversion efficiency and larval performance, followed by brewer's spent grain and potato sorting residues, whereas potato peels induced substantial mortality (57%). Substrate reduction ranged from 34 to 55%, with okara again yielding the highest rate. Different substrate dependent gut microbial community were detected, yet Enterococcaceae consistently dominated, albeit with reduced relative abundance in larvae fed with potato peels and potato sorting waste. These to latter substrates promoted also higher bacterial loads compared with the standard diet. Bacterial supplementation did not influence larval survival or final biomass yield, although significant differences in larval weight emerged after the second inoculation, particularly with active strains. Overall, the exploitation of agri-food by products represents a valuable strategy to enhance the sustainability of black soldier fly production. Further insights are required to elucidate how substrate influence the gut microbiota and, in turn, insect–microorganism interactions, ultimately supporting the development of probiotic assisted rearing strategies to optimise insect rearing and its health. Acknowledgement: Work funded by PRIN 2022 Project InProFarm (Insect Probiotic-assisted Farming: a promising tool to enhance edible insect health and performances), grant number 2022L4NJMK-Next Generation EU, Mission 4, Component 2.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Evaluating the effect of chemical pretreatment of lignocellulosic biomass through black soldier fly larvae performance

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Lignocellulosic biomass (LCB), composed mainly of cellulose, hemicellulose and lignin, is the most abundant renewable resource with potential applications for bioconversion. Recalcitrant structure limits LCB's biological utilisation. Chemical pretreatment (C-PT) reduces covalent and hydrogen bonds, crystallinity and the degree of polymerization, thereby increasing cellulose accessibility, disrupting lignin-cellulose and lignin-hemicellulose complexes. This study investigated the effect of C-PT of LCB on black soldier fly larvae (BSFL) performance, including survival rate (SR), larval growth (LG), bioconversion rate (BCR), efficiency of conversion of ingested feed (ECI), and feed conversion ratio (FCR). LCB substrates were pretreated with aqueous solutions of 1.0% and 2.0% NaOH and 1.0% and 2.0% citric acid (CA), separately, defined as A, B, C and D, and untreated substrate as control diet (CRL). One hundred BSFL were raised on untreated and treated substrates in plastic containers (diameter 10–14 cm, height 8 cm) for 14 days with three replicates. Rearing conditions: 28.5 ± 0.2 °C, RH 56.2 ± 3.2%, feeding rate 50 mg/larva/day, feed moisture 85%. SR exceeded 85% in all trials. The highest SR (98.67 ± 0.88) and LG (78.04 ± 2.48 mg larva-1) were determined for BSFL reared on A compared to CRL (95.67 ± 1.76% and 23.48 ± 0.38 mg larva-1, respectively). ECI (%) = 12.23 ± 1.20 (CRL); 44.89 ± 2.99 (A); 54.84 ± 5.61 (B); 35.36 ± 7.41 (C); and 32.52 ± 1.58 (D) as well as BCR (%) = 3.31 ± 0.06; 11.25 ± 0.36; 5.63 ± 1.03, 8.21 ± 0.69; and 9.32 ± 0.17 for BSFL, while FCR (mg larva-1) = 8.35 ± 0.88; 2.25 ± 0.15; 1.87 ± 0.21; 3.07 ± 0.60 and 3.09 ± 0.16, respectively. Increased LG, ECI, and BCR as well as reduced FCR compared to CRL indicate substrates become more convertible into BSFL-biomass upon C-PT. LG and BCR (except CRL vs 2.0% NaOH (B)), ECI (except CRL vs 2.0% CA (D)) and FCR are statistically significant ($p < 0.05$) compared to CRL. In conclusion, C-PT improves LCB quality for BSFL cultivation, supporting sustainable waste valorisation and circular bioeconomy strategies. Acknowledgement: Funded by Volkswagen Foundation.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

The effects of fish-faeces diets on the growth and elemental composition of *Hermetia illucens* and *Tenebrio molitor*

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The potential use of aquaculture sludge as a substrate for the black soldier fly (BSFL) has been investigated in various studies, and this use is usually associated with the risk of accumulating heavy metals in the larval biomass produced. In the current study, pure fish faeces, not contaminated with fish feed or fish carcass, was included in the substrates of BSF and the yellow mealworm. The substrates contained chicken feed and straw, and the chicken feed was replaced with fish faeces. Growth performance, fresh and dry yield, crude protein content, feed conversion ratio (FCR), survival rate, and elemental composition were measured. The results showed that even a 19% inclusion of fish faeces did not impair BSFL performance indicators, while dry yield and FCR were optimal at 15%, inclusion and survival rates remained high across all treatments. Elemental analysis revealed increasing concentrations of various elements (e.g., Fe, Zn, Cu, Pb, Cd) the higher the inclusion of fish faeces is; however, the levels of As, Cd, Pb, and Hg remained at or below current EU maximum limits for feed materials, with only Cd nearly reaching the limit for complete feed at the highest inclusion rate. The yellow mealworms (MW) were fed the same diet and the same parameters were assessed. Growth performance did not differ among treatments regardless of fish faeces inclusion level, and the concentration of most measured elements increased with the inclusion rate. However, accumulation of all examined elements was markedly higher in BSFL than in mealworms. Overall, the results indicate that both insect species can be fed diets containing up to 19% or moderate levels of fish faeces without impairing larval growth. MW biomass appears to be "safer" if the goal is to produce insect biomass with lower concentrations of the undesirable heavy metals.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Developing cost-effective enzymatic aqueous oil extraction for industrial BSF biorefineries: preliminary results

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The black soldier fly (*Hermetia illucens*, BSF) is a key species for emerging insect biorefineries due to its capacity to convert low-value organic substrates into high-value lipids, proteins, and bioactive compounds. To enhance sustainability and industrial scalability, alternatives to conventional solvent-based oil extraction are required. Aqueous enzymatic extraction (AEE) represents a promising solvent-free method with lower environmental impact and improved product quality, suitable for food, feed, and other refined applications. This study evaluated the use of Alcalase (ALC) from *Bacillus licheniformis* and Protease (PROT) from *Bacillus* sp. at 0, 0.4 and 2% (enzyme:dry larval biomass) for aqueous oil extraction from BSF larvae reared on olive pomace, a common agro-industrial by-product in Portugal. Following pH adjustment, incubation at 55 °C for 1h45min, centrifugation, and oil recovery, oil yield (%) and extraction efficiency (%) were determined. Both parameters increased consistently with enzyme concentration. Yield ranged from 17.70 to 19.79% (mean ± SD = 18.95 ± 1.02%, and extraction efficiency from 51.56 to 56.95% (mean ± SD = 54.34 ± 2.94%). For ALC, yield increased from 17.70 ± 0.71% to 19.55 ± 1.40% and efficiency from 51.56 ± 2.07% to 56.95 ± 4.09%, whereas for PROT yield rose from 18.35 ± 0.33% to 19.79 ± 0.68% and efficiency from 51.80 ± 0.92% to 55.88 ± 1.93%. No significant differences were observed between enzymes, and no enzyme–concentration interaction was detected, although enzyme concentration showed a trend toward higher yield ($p = 0.056$) and efficiency ($p = 0.058$). While oil yields remain lower than those typically achieved by solvent extraction (30–40%), AEE offers advantages in sustainability, safety, and product quality. Future work will focus on process optimisation (enzyme dosage and reaction conditions), enzyme recycling across multiple extraction cycles, and full valorisation of residual fractions (oil, proteins, chitin, and other bioactive compounds) to enhance economic and environmental feasibility. Process robustness will also be assessed using BSF larvae reared on diverse biowaste substrates. This integrated, solvent-free approach supports circular bioeconomy principles by coupling biowaste valorisation with high-value biorefinery outputs and cost-efficient processing.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Spent coffee grounds and their mode of drying as feed ingredient for *Tenebrio molitor*

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Spent coffee grounds (SCG) are a major by-product of the coffee industry that remains underutilised despite being rich in interesting bioactive compounds with health properties. This study evaluated the potential valorisation of SCG through their inclusion in the diets of *Tenebrio molitor* larvae, assessing growth performance, survival, and larval nutritional composition under different inclusion levels and drying processes of SCG. Experimental diets were formulated using wheat bran as a control (C) or partially replaced with SCG. Freeze-dried SCG were included at 15, 30 and 50% (FD15, FD30, FD50) to evaluate larval tolerance to increasing inclusion levels, while oven-dried SCG were tested at 15% (OD15) to compare drying methods at the same inclusion rate. The trial was performed in triplicate (845 larvae, 4 weeks-old, 8.6 mg/larva, 210 mg feed/larva) at 27 °C, 60% RH, and water supplied as agar, until 12 weeks-old. Mortality, weight gain (WG) and mass reduction (MR) were estimated. Larvae were blanched (100 °C, 1 min), oven-dried (60 °C, 18 h), and analysed. Larvae fed the control diet exhibited WG of 103.9 mg/larva. SCG inclusion induced a dose-dependent decline in larval growth, influenced by the drying process of SCG. Thus, inclusion of freeze-dried SCG above 15% led to severe growth impairment (WG <10 mg/larva) and mortality (72–97%). At the same inclusion level (15%), oven-dried SCG resulted in WG value around 40% higher than those observed for freeze-dried SCG. Nevertheless, oven-dried SCG still resulted in around 40% lower larval growth than control (62.5 mg/larva). At 15% inclusion, MR also decreased with SCG treatments, particularly for freeze-dried SCG. Despite reduced growth, SCG increased protein content in larvae (36, 41 and 51% for C, OD15 and FD15, respectively), and reduced lipid levels (36, 20 and 11 for C, FD15 and OD15, respectively). This preliminary assay demonstrates that SCG, its drying method and inclusion level affects *T. molitor* rearing performance and composition. While reduced growth and survival suggest the need for caution, the notable larval nutritional improvement, by protein enrichment and lipid reduction, supports further research on optimised SCG processing and inclusion strategies, as well as further exploration on the potential changes in the functional properties of insect biomass. Acknowledgment: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Conservation of organic solid waste and agro-industrial by-products for bioremediation with black soldier fly larvae

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The inadequate management of organic solid waste (OSW) and agro-industrial by-products is an environmental and economic challenge. Their valorisation through bioremediation with black soldier fly *Hermetia illucens* (BSF) larvae is a sustainable alternative, but its efficiency depends on the preservation of the substrate. This study aimed to investigate the conservation of OSW and by-products zucchini (CUR) and broccoli stalks (TB) over 45 days, combining packaging type (plastic and vacuum), temperature (ambient and refrigeration at 4 °C), and the use of a preservative (0.5% citric acid). For each substrate, the following treatments were applied: (1) plastic bags at ambient temperature (SPAmb); (2) plastic bags under refrigeration at 4 °C (SPRef); (3) vacuum bags at ambient temperature (SVAmb); (4) vacuum bags under refrigeration at 4 °C (SVRef); (5) preservative in bag at ambient temperature (CVAmb) and (6) preservative in bag under refrigeration at 4 °C (CVRef). Chemical composition and nutritional losses were analysed, and statistical analysis was performed using PROC MIXED procedure of SAS (Statistical Analysis System). The CV treatments showed better stability of dry matter (DM) and non-nitrogen extractives (NNE) (values above 480 and 670 g/kg DM in OSW and by-products, respectively), indicating effective inhibition of microbial degradation, although with higher losses of crude protein (CP) (up to 38.8 and 25.4% in CUR and TB, respectively) and crude fat (CF) (83.4 and 57.5% in CUR and TB, respectively). In contrast, the SP and SV methods better preserved protein and fibre but resulted in higher losses of NNE (up to 24.4% in OSW and 27.4% in by-products). Refrigeration reduced some losses, but the preservation method was the key factor. Overall, preservation with citric acid optimises substrate stabilization for BSF larvae, while plastic and vacuum methods may be preferable when the primary goal is protein-rich larval biomass.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Composting of *Tenebrio molitor* frass to reduce phytotoxicity: implications for agronomic use as an organic fertiliser

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The growing interest for insect farming for food and feed production is generating increasing amounts of insect frass, a by-product with considerable potential as an organic fertiliser. Previous studies by the authors have shown that the soil application of frass can stimulate soil biological activity, increasing microbial biomass C and respiration by 4 and 20 times compared to the control, respectively, as well as double the availability of mineral N. Such effects have been attributed both to the stimulation of native soil microorganisms and to the direct input of exogenous microbial biomass contained in the frass itself. Anyway, the agronomic use of frass is often constrained by phytotoxic effects. This study evaluates composting as a practical post-treatment to reduce the phytotoxicity of *Tenebrio molitor* (TM) frass and to improve its suitability for agricultural applications. TM colony was reared in plastic trays (60 × 40 × 13 cm) at 1 larva/cm² rearing density in a climate-controlled chamber (T = 26 ± 1 °C, RH = 55 ± 3%, 10L:14D) at the University of Udine. Wheat bran was used as substrate, and potato slices were added every 4 days as a water source. TM frass was composted for 30 days under controlled moisture (60%) and aeration conditions. A germination trial was conducted using maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) grown on soil amended with composted frass (CF) or untreated frass (UF) at two application rates (4% and 8%, w/w), alongside an unamended control. Plant responses were assessed after 15 days by measuring fresh weight (FW), dry weight (DW) and germination index (GI), followed by two-way ANOVA. UF significantly reduced plant performance, particularly at the 8% rate, with GI values indicating phytotoxic conditions. Composting improved frass performance, increasing GI values to non-phytotoxic or stimulating classes depending on crop and dose. In maize, CF applied at 4% resulted in GI values above 80% and significantly higher FW and DW compared to UF, with whole-plant FW exceeding the control, in some cases. In sunflower, composting reduced phytotoxicity at both application rates, although biomass responses were more variable. Overall, composting reduced without eliminating frass phytotoxicity, with the 4% application rate consistently outperforming the 8% rate. When combined with the stimulation of soil microbial activity and nutrient availability, these results support composted TM frass as a promising organic fertiliser once appropriate post-treatment is adopted to safely integrate insect farming by-products into sustainable agricultural systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Investigating black soldier fly *Hermetia illucens* frass fertility: from crop residues to insect feed and bio-fertilisers

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Edible insects are increasingly grown in Canada for pet and livestock protein supplements, and to digest and manage food waste streams. Mass-reared insects like black soldier fly (BSF) are used in the bioconversion of food waste, including fruits and vegetables, to protein and frass. Other available food grade plant residues include field crops (edible bean, soybean, corn) and cover crops (buckwheat, mustard, peas) that have minimal pesticide use. Trials with various crop residues and seeds from cover crops indicated that BSF had 100% survival up to the pre-pupal stage (11 days) and larval weights were similar to those fed the control diet. Typically, the materials that produced the higher BSF weights, for example buckwheat (BW) seed, had higher carbohydrates (20%), but less crude protein (20%) than the control feed, Gainesville diet (GVD). Promising crop wastes were corn red dog (kernel chaff/dust), soybean hulls, and edible bean husks and stems. A combination of BW seed with less nutritious residues, such as dried distillers grain (DDG) at 40:60 BW:DDG, increased the weight of BSF to that of the GVD-fed larvae by the prepupal stage. Insect frass produced by BSF from the crop residue diet can also provide a source of fertiliser for growing vegetables and field crops. Field trials with frass produced by a Canadian commercial BSF supplier determined that recommended chemical fertiliser rates could be reduced if 500 to 1000 kg/Ha frass contributed part of the N-P-K. The growth (biomass, plant density, canopy area) of cover crops, BW and brown mustard (BM), was no different between fertiliser alone and frass-fertiliser combined. For example, 48.2% less N, and 100% less P and K were needed to grow BW when 1000 kg/Ha frass was applied. With BM, 28.4% less N, 60.9% less P, and 75.9% less K were required. The benefit of harvesting the BW seed for use as BSF feed was that the soil nutrients (N-P-K) were not significantly different between the time when cover crops are terminated (before seed set) and the time required for seed harvest (2-4 weeks later). Measurement of contaminants in the frass from these promising feed sources indicated that heavy metals met the standards for use as a fertiliser. These findings suggest that selected field crop residues and cover crops, singly or in combination, can: (1) provide nutritious feed for BSF; (2) reduce crop wastes; (3) provide a rich fertiliser for cover crop growth; (4) reduce chemical fertiliser use and (5) constitute key components in a novel circular economy. Future studies will examine the application of frass produced from mixtures of the field crop wastes as an amendment for cover crop growth and seed yield.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Valorisation of agricultural by-products as nutritional substrates for *Tenebrio molitor* larvae

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The utilisation of locally produced agricultural by-products as a feed source for *Tenebrio molitor* larvae offers numerous advantages and contributes to the development of sustainable circular economy models. The study aimed to evaluate the impact of various by-product-based diets on larval growth, survival, Efficiency of Conversion of Ingested Food (ECI) and on the nutritional profile of yellow mealworm (MW) larvae. Homogeneous larval populations were obtained by collecting eggs from peak-reproduction adults of the stable colony of the University of Udine. Experimental substrates were formulated using pea (P), buckwheat (BW), barley (B) and soyabean (S) as 50% replacement to the high-value wheat bran (WB) rearing substrate (CTRL). Groups of 50 28-day larvae (1.99 ± 0.05 mg) per replicate (5/substrate) were transferred to a plastic box (17.5 × 9.3 × 6.3 cm) with aeration slits on the top according to a completely randomised design and maintained at 27 °C and 60% RH. Larvae were bulk weighed at the beginning and during the experimental period. The MW grew in all the substrates and MANOVA revealed a significant effect of the substrate on the following combined production variables: single-larva weight and their survival at the day of first pupal appearance, fresh yield, and pupation time ($p < 0.05$), but not on the pupal weight (119 ± 19 mg). The mean single-larva weight resulted the highest in CTRL (122 ± 13.7 mg) and BW followed by S, PP and the lowest in B (47 ± 8.9 mg). The highest larvae survival rate was observed in the CTRL (98%), followed by S, BW, PP (70%). The barley-based diet (B50) showed significantly lower survival (47%), with most mortality occurring in the first 20 days. Time of pupation differed with values ranging from 84 days (CTRL) to 154 days (B). Substrate significantly affected ECI (% DM): the highest value was registered in BW (58 ± 8.5) and lowest values in B50 (15.4 ± 4.6) ($p < 0.05$). Rearing substrate significantly affected larvae DM, protein (Kjeldhal N*4.76), lipid, ash and chitin content (ADF-ADL) ($p < 0.05$) as well as the fatty acids and mineral composition. The research shows that specific agri-food by-products like buckwheat, soy and pea can be effectively integrated into *T. molitor* diets, thereby supporting the transition to more sustainable and circular food systems while suggesting the possibility to tailor products for specific targets of consumers. Acknowledgement: This work was supported by the University of Udine in the framework of the Strategic Plan 2022-25 Interdepartmental Research Project CibiAmo.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Bioaccumulation and fate of per- and polyfluoroalkyl substances (PFAS) in black soldier fly (*Hermetia illucens*) larvae

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The black soldier fly (*Hermetia illucens*) industry is an evolving and expanding sector globally, driven by its capacity to upcycle diverse organic matter into valuable products like larval biomass and frass. Previous work in our lab has demonstrated that digestates from anaerobic digestion originating from industrial plants can be used as a feeding substrate for black soldier fly larvae when an energy source is supplemented, thereby enabling the valorisation of the other nutrients present. However, using waste substrates, such as anaerobic digestates, poses contamination risks from trace elements, agrochemicals, antibiotics, microplastics and persistent toxins, including per- and polyfluoroalkyl substances (PFAS): synthetic "forever chemicals" known for environmental persistence, bioaccumulation, and health impacts at low concentrations. This study assessed PFAS dynamics during larval bioconversion of solid digestates from an urban organic waste biomethanisation plant, primarily processing wastewater treatment sludges mixed with household organic residuals. Up to 80 PFAS compounds were analysed in digestates (feed), larvae, and frass using LC-MS/MS at two independent laboratories (Université de Montréal and SGS-Axys), finding the same dynamics in both. Total PFAS concentrations in digestates were 36.5 and 47.9 μg/kg dry weight (dw), dominated by perfluorooctane sulfonate (PFOS; 33.2 and 32.4 μg/kg dw). Post-bioconversion, PFAS distributed to larvae (179 and 200 μg/kg dw) and frass (25.9 and 40.4 μg/kg dw), indicating bioaccumulation in larvae (e.g., PFOS up to 189 μg/kg dw) and a reduction of 16-67% in frass for key compounds such as PFOS and PFHxA. In absolute terms, input PFAS (6 and 7.9 μg per unit) partitioned to larvae (1 and 1.1 μg) and frass (4.4 and 7.1 μg), suggesting minor losses, transformations or external inputs according to specific PFAS (e.g., reductions in PFHxA and NEtFOSE, increases in PFNA and PFDoA). This dynamic depends on several factors but shows that some of the PFAS are being transformed during the process. These findings demonstrate PFAS bioaccumulation in larvae, with potential frass decontamination, highlighting opportunities for defining bioremediation strategies in waste management.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Circular use of olive waste (OLIWA) via black soldier fly rearing for feed, biopackaging and biogas

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Olive oil production results in waste and by-products (OBs) containing bioactive compounds. The OLIWA project aims to create a circular economy model applicable at the local level and to reduce food loss and waste across six Mediterranean countries (Italy, Spain, Greece, Turkey, Algeria and Tunisia). More specifically, the project will explore OB's use in insect rearing, investigate the effects of olive natural extracts (NE), insect meals and their association on the intestinal barrier by means of in-vitro testing, investigate the effects of NE and insect meals on animal models, poultry and fish, trying to enhance gut health and final product quality, investigate OB's potential in food packaging and use insect frass, poultry manure and olive waste for biogas production to be used in local industries. By proposing insects as an alternative source of proteins for animal feed and utilising OBs for insect meal production, OLIWA addresses the EU's need for sustainable food security and reduced reliance on imported feeds. Furthermore, producing new active bio packaging material will prolong the shelf life of animal products reducing food waste and improving the product quality. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

From waste to wealth: engineering black soldier fly bioconversion for a circular waste economy in Saudi Arabia

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Saudi Arabia faces a dual challenge of increasing organic waste generation and dependence on imported feed and fertilisers. Approximately 33.1% of available food is lost or wasted across the supply chain, while per-capita municipal solid waste generation average 1.72 kg person a day between 2010 and 2018. Municipal solid waste management costs are projected to exceed USD 10 billion annually, and food waste is estimated at 4.07 Mt yearly, intensifying pressure on food security and waste management systems. Addressing these challenges aligns with the Kingdom's Vision 2030 priorities for sustainability, circular resource use, and local value creation. At KAUST, we developed a modular and easily deployed black soldier fly (*Hermetia illucens*) bioconversion system designed to valorise heterogeneous organic waste streams under arid climatic conditions. The modular architecture enable scalable, rapid deployment across municipal and agro-industrial sectors. A pilot facility processing up to 5 t/day of mixed municipal and agro-industrial waste in the Thuwal region demonstrates the scalability of this waste-to-resource approach. Substrate specific feeding trials quantified feed conversion ratio (FCR), biomass yield, nutrient recovery, and environmental performance. Larval biomass achieved up to 47% crude protein (dry weight), with FCR values ranging from 1.6 to 1.9, depending on substrate composition. Amino acid profiling and the vitro digestibility confirmed suitability for aquaculture and poultry feed formulations. Organic waste mass was reduced by 62–71% within a 10–12-day larval growth cycle. Residual frass was thermochemically upgraded into a biochar-enriched soil amendment, increasing soil water holding capacity by 28–35% and improving controlled nutrient release in sandy soils. Extracted larval lipids (up to 32% dry weight) exhibited favourable fatty acid profiles for biodiesel and feed applications, while purified chitin yielded chitosan with up to 85% deacetylation efficiency. Anaerobically digested sewage sludge evaluated as a substrate resulted in substantial reductions of *Escherichia coli* and *Salmonella* spp., producing an organic fertiliser compliant with national regulatory thresholds. These results demonstrate the technical feasibility and regulatory readiness of BSF-based bioconversion into Saudi's municipal waste management. The modular platform delivers co-benefits in sustainable feed production, soil rehabilitation, biosolid stabilization, and localized resource recovery, supporting a scalable circular bioeconomy for arid environments.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Productivity of *Tenebrio molitor* adults on different by-products

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Thanks to their ability to convert organic matter into nutritious biomass, insects can help solve the issue of food waste and loss. *Tenebrio molitor*, the yellow mealworm, is one of the most studied species for the bioconversion of agro-industrial by-products. According to most current research, our primary focus is the evaluation of various byproducts (tomato peels, potato waste, brewer's grain, okara, hemp waste, and wheat bran as control diet) for their ability to support the growth of yellow mealworm larvae. However, diet also has a profound impact on the lifespan, growth and productivity of adults. The reproductive phase and tray-based production are fundamental aspects of mass rearing. This study aims to evaluate the effectiveness of by-products, previously tested for their influence on larval development, on the oviposition. Newly emerged adults, sexed during the pupal stage and emerged from different substrates, were used for the experiments. Two different tests were set up: I) one adult couple per replicate; II) four adults couples per replicate. After ten days, adults were removed from each replicate for seven times (total of 70 days), and moved to another box with the same substrate. The tests were observed weekly, and once the new larvae in containers were visible, they were counted. Potato waste was the worst substrate, allowing the emergence of very few adults. Similar trends were observed in both experiments with one or four couple of yellow mealworm. Overall, higher number of larvae were observed on wheat, hemp waste and tomato peels (wheat: 29.33 ± 2.63; hemp waste: 22.46 ± 1.59 and tomato peels: 21.70 ± 0.77). A significantly lower number of larvae was observed on okara and brewer's grain (respectively, okara: 5.23 ± 0.96 and brewer's grain: 6.03 ± 1.09). When observing the egg-laying time, significant differences were only observed between the wheat and hemp waste, with a significantly higher presence of larvae developing between 30 and 40 days for the test with single couple. With four couples, no differences were found. Tests on okara and brewer's grain revealed a large number of dead small larvae, indicating that oviposition was possible but probably not favourable for larval growth. In fact, number of offspring or productivity may not always be related to female fecundity, but rather to larval mortality after egg hatching. Conversely, hemp waste and tomato peels proved suitable for both larval growth and oviposition, which is an important requirement for efficient mass rearing. Acknowledgement: Research funded by the EU project "ON Foods – Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security — Working ON Foods" (NRRP, No. 1550).

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Antimicrobial extracts from edible insects: modulation through olive oil by-product-enriched diets

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Supplying insects with by-products rich in antimicrobial compounds may enable to valorise side streams, while enhancing the antimicrobial activity of insect-derived products through bioaccumulation of such dietary compounds. The aim of this study was to assess the impact of dietary supplementation of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) larvae with by-products rich in antimicrobial compounds, such as olive by-products (olive leaves and olive pomace) on the subsequent antibacterial activity of extracts obtained from the fed larvae. Larvae of both species were reared a control diet (wheat bran), or wheat bran replaced with 30% olive leaves or 30% olive pomace. After rearing, larvae were slaughtered, dried, ground, and defatted. Bioactive extracts were obtained by ultrasound-assisted extraction with 70% ethanol from defatted flours. Antibacterial activity of the insect extracts was evaluated by broth microdilution against six bacterial species, including three Gram-positive (*Staphylococcus* aureus, *Listeria monocytogenes*, and *Bacillus subtilis*) and three Gram-negative (*Escherichia coli*, *Salmonella* spp. and *Campylobacter jejuni*) bacteria. TM extracts lacked significant antibacterial activity against most tested bacteria, except for *C. jejuni*, for which larvae fed olive pomace showed a 1.22 log reduction in growth. In contrast, HI extracts exhibited strong antibacterial effects against the Gram-positive strains (1.56–5.82 log reduction), regardless of the diet. It was also remarkable that extracts obtained from larvae fed olive oil by-products showed a strong antibacterial effect (>6 log reduction) against *S. aureus* and *B. subtilis*, compared to extracts from control larvae. These findings indicate that both insect species and diets modulate the antibacterial activity of extracts obtained from insect flours. Overall, the study highlights the potential to produce tailored antibacterial extracts from *H. illucens* larvae reared on specific olive by-products as promising ingredients for health- and safety-related applications within a circular economy framework. Acknowledgment: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Effects of heavy metal exposure on *Hermetia illucens* larvae

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The larvae of the black soldier fly (BSFL), *Hermetia illucens*, are highly efficient bioconversion agents of a wide range of organic waste. The possibility to tap into new rearing substrates for BSFL not yet authorised by EU legislation could allow increasing production volumes and reducing the price of BSF meal. In this setting, the organic fraction of municipal solid waste (OFMSW) is one of the most promising wastes to be used due to its high nutritional value and high quantities. However, the use of these substrates, that are considered unsafe due to the possible presence of chemical and microbiological hazards, may expose BSFL to various pollutants, including heavy metals (HMs), that could potentially compromise larval growth and health. Moreover, the possible accumulation of these xenobiotics in larval organs and tissues may pose a risk for BSFL-derived products. In this study we reared BSFL on OFMSW contaminated with two concentrations of $PbCl_2$ (i.e., 30 mg/kg and 120 mg/kg, d.m.). Growth performance, survival rate, and bioconversion efficiency were monitored and compared with control larvae fed on OFMSW devoid of lead. In addition, to evaluate the effects of this HM on larval physiology, the activation of the immune system was monitored through cellular (i.e., haemocyte counts) and humoral (i.e., antimicrobial activity) markers. Finally, lead accumulation was quantified in whole larvae and in different body tissues, as well as in the frass. Our results demonstrate that, although no effects on life cycle and development were observed — as confirmed by comparable survival rate and maximum weight reached by BSFL across different treatments — exposure to high lead concentrations induced the activation of both cellular and humoral immune components. In addition, we detected the accumulation of lead in various larval tissues (including the midgut epithelium, fat body, and Malpighian tubules) and in frass and whole larvae, underlining the potential risk associated with the use of these larvae for the preparation of BSFL-based products. This study provides important insights into the effects of HM contamination on BSFL, highlighting the need for rigorous safety evaluations when using BSFL to bioconvert contaminated substrates for production purposes.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

What is the phytohormone content of *Hermetia illucens* frass?

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Organic fertilisers produced via bioconversion are an important element of sustainable, cleaner agricultural systems. In this context, insect frass has gained attention as a promising biofertiliser because it is nutrient-dense, harbours beneficial microbiota, and can promote plant growth. Nevertheless, the phytohormone profile of frass generated by *Hermetia illucens* (black soldier fly, BSF) larvae is still poorly described (Green 2023; Lopes *et al.*, 2025). Here, we present a comprehensive evaluation of the three major phytohormone groups in BSF frass and associated leachates obtained during the bioconversion of pea and bean seed waste. These plants, belonging to legumes, are important annual agronomic crops grown primarily for their protein-rich seeds. According to FAO, legume production reached about 9.6 billion tons worldwide in 2022, and projections suggest it will rise to roughly 125 million tons by 2032 (FAO,2023). Yet industrial processing of the seeds produces substantial residues, typically accounting for approx. 5–25% of the initial raw material mass (Karaca and Nickerson, 2022). Using *H. illucens* larvae we bioconverted very low-quality pea and bean seed waste, which cannot be use for feeding others animals and then determined phytohormones in it. Both substrates enabled efficient larval development. Frass from bean waste contained higher amounts of indole-3-acetic acid, stress-associated hormones, and the total cytokinin pool, whereas leachate from pea frass showed increased levels of trans-zeatin, cis-zeatin, and IP. IAA and selected cytokinins were also identified in the larvae. Overall, our results indicate that BSF-driven bioconversion improves the phytohormonal profile of frass more effectively than microbiota-only decomposition, thereby strengthening its prospects as a multifunctional, environmentally friendly biofertiliser. This work highlights how insect-based bioconversion can upgrade agri-food residues by adding functional value within sustainable production frameworks. References: FAO, 2023. Agricultural production statistics 2000–2022. FAOSTAT Analytical Briefs, No. 79. FAO, Rome. Green, T., 2023. A biochemical analysis of black soldier fly (*Hermetia illucens*) larval frass plant growth promoting activity. PLoS One 18: e0288913. Karaca A.C. and Nickerson M.T., 2022. Developing value-added protein ingredients from wastes and byproducts of pulses: challenges and opportunities. ACS Omega 23: 18192-18196. Lopes I.G., Gómez-Brandón, M., Praeg, M., Claeys, J., Yakti, W., Bitterlich, M., Jones, J.J., Geilfus, C.M. and T. Klammsteiner, T., 2025. BugBook: Critical considerations for evaluating and applying insect frass. Journal of Insects as Food and Feed 11: S507-S532.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Valorisation of olive oil by-products in Algeria using the black soldier fly

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In recent years, Algeria's olive oil sector has grown significantly due to national programs. Olive groves now cover over 440 000 ha, producing 70 000–100 000 tonnes annually, making olive cultivation a key driver of rural development and the agri-food sector. The increase in production generated substantial quantities of organic by-products, including liquid effluents and solid residues such as olive pomace and olive mill wastewater, characterised by a significant content of phenolic compounds with phytotoxic activity, which pose serious environmental challenges in the absence of appropriate valorisation pathways. In this context, the black soldier fly (BSF) emerges as an innovative biotechnological solution for the bioconversion of low-value organic substrates. Its larvae exhibit a strong capacity to transform complex matrices into a protein- and lipid-rich biomass, which can be used as a sustainable source of alternative proteins for animal feed. Beyond larval biomass, BSF by-products, such as larval cuticle and exuviae, represent a promising source of chitin. This biopolymer has attracted increasing interest in pharmaceutical, biomedical, and biomaterials applications due to its biocompatibility and biodegradability. In addition, the frass generated during larval rearing constitutes a valuable organic amendment that can improve soil fertility and biological activity. This research highlights the strategic potential of integrating BSF into the management of olive oil by-products in Algeria, in line with the principles of circular bioeconomy and zero-waste valorisation. Although BSF farming remains poorly structured in Algeria, it shows strong development potential and deserves further attention. The PRIMA OLIWA project aligns with this objective by aiming to demonstrate the technical and environmental feasibility of integrating BSF into circular valorisation systems for olive oil co-products in the Mediterranean context.
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INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

From waste to resource: agri-food by-products as substrates for *Tenebrio molitor* rearing

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Steady population growth and the increasing global demand for protein require the identification of sustainable alternative sources. Insect farming is widely recognized as a promising solution, particularly when integrated into a circular economy framework. Among major agrobusiness, tomato processing and brewery production generate by-products accounting for approx. 10–30% of the total raw material processed, which contain valuable nutritional components that make them attractive substrates for insect rearing. This study evaluated the suitability of two by-products as feed source for the yellow mealworm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). Experiments were conducted separately on two genetically distinct yellow mealworm populations, originating from Milan and Turin. Dried tomato peels and brewer's spent grain were mixed with wheat bran (1:1, v/v) as growth substrates and were provided along with wheat bran as control treatment. Development time, survival, larval biomass, individual weight and individual length were assessed. For the Milan population, no significant differences were observed between diets in terms of survival (≈95%), total larval biomass, or development time (34 days to first pupation). However, larvae reared on the tomato peel mixture showed a significantly higher mean individual weight and length (0.144 ± 0.004 g; 27.01 ± 0.23 mm) compared to the control (0.096 ± 0.003 g; 22.88 ± 0.28 mm). For the Turin population no significant differences were observed between diets in terms of survival (≈89%), and total larval biomass. Differences were found in development time between brewer's (118 days) and control diet (50 days). Larvae reared on brewery's mixture showed a significantly lesser mean individual weight and length (0.075 ± 0.002 g; 19.41 ± 0.22 mm) compared to the control (0.085 ± 0.002 g; 21.48 ± 0.19 mm). Overall, these findings support the potential of tomato peels and brewer's spent grain as sustainable substrates for yellow mealworm mass rearing. Nevertheless, deformities observed at late larval and pupal stages with tomato peels highlight the need for further investigations on adult reproductive performance and on the possible presence of pesticide residues or anti-nutritional factors, emphasizing the importance of careful selection and quality control of by-products as insect feeding. Acknowledgement: Work funded by PRIN 2022 Project InProFarm (Insect Probiotic-assisted Farming: a promising tool to enhance edible insect health and performances), grant number 2022L4NJMK — Next Generation EU, Mission 4, Component 2.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Growth performance and microbial composition of black soldier fly larvae fed different food waste substrates

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Despite increasing food insecurity, >30% of the global food supply is wasted, contributing substantially to greenhouse gas emissions. Black soldier fly larvae (BSFL) composting offers a circular solution by converting food waste into insect biomass for animal feed and nutrient-rich frass for biofertilisation. However, diet-specific impacts on larval performance, nutrient transformation, and microbial composition remain insufficiently characterised. This study evaluated BSFL growth, feed conversion, nutrient composition, and associated microbial community dynamics across four food-waste substrates: cooked chicken, fruits and vegetables, starch-rich foods, and a mixed diet combining all categories. Food waste was fed to BSFL over a 14-day period. Larval growth was assessed by changes in length, width, and mass on days 1, 7 and 14, and feed conversion ratios (FCR) were calculated. Proximate composition of feedstocks and larvae was determined. Microbial abundance and community structure in food waste and frass were analysed using 16S rRNA amplicon sequencing. Statistical differences were evaluated using ANOVA with Tukey's HSD ($\alpha = 0.05$). Larvae fed exclusively on chicken exhibited significantly reduced growth, failing to reach the prepupal stage, with final mass 33% lower than those reared on fruit/vegetable waste and 56–62% lower than starch-rich and mixed diets. Starch-rich and mixed diets achieved the highest biomass gain (6.8–7.9 mg/larva) and most efficient feed conversion (FCR = 2.9–3.2). Although chicken-fed larvae accumulated the highest protein content (26.7 ± 0.5%), inadequate carbohydrate availability limited development. Balanced macronutrient profiles were observed in larvae reared on starch-rich and mixed substrates. Microbial analyses revealed strong diet-driven effects. Chicken waste exhibited the highest bacterial abundance (8×10^{12} gene copies/g) but lowest alpha diversity (Shannon index), dominated by lactic acid bacteria. Fruit/vegetable and mixed wastes showed 38–40% higher alpha diversity. Following digestion, starch-derived frass exhibited the highest bacterial load, while mixed-waste frass showed marked selective enrichment dominated by *Atopostipes suicloacalis* (65.3%) and *Vagococcus lutrae* (21.1%). Beta-diversity analyses confirmed distinct, diet-specific microbial communities that persisted post-digestion. Overall, diversified, carbohydrate-rich food waste optimised BSFL growth, bioconversion efficiency, and microbial restructuring, supporting the scalability of BSFL-based systems for sustainable food waste management and tailored biofertiliser production.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

The giving gut: valorisation of insect residues via insect gut microbiome fermentation for postbiotic production

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Postbiotics, defined as preparations of inactivated microorganisms and/or their components that confer functional benefits in biological systems, are emerging as safe and stable alternatives to live probiotics for applications in food, feed, and biomanufacturing. Insect gut microbiomes represent a largely untapped reservoir of enzymatic and metabolic diversity capable of converting low-value agro-industrial residues into functional postbiotic preparations. In this study, gut microbial consortia from selected insect species were used as biocatalysts to valorise lignocellulosic and insect-derived substrates, including corn stover and insect frass. Shotgun metagenomic analysis revealed diverse microbial communities enriched in genes encoding carbohydrate-active enzymes, including cellulases, chitinases, and proteases, as well as pathways associated with short-chain fatty acid and amino acid metabolism. Electron-beam irradiation was applied as a non-chemical pretreatment to enhance substrate accessibility while maintaining a clean-label process. Subsequent anaerobic fermentation yielded metabolite-rich fractions, including short-chain fatty acids, organic acids, and bioactive peptides. Post-fermentation inactivation yielded sterile postbiotic preparations, which were fractionated into cell-free supernatants and microbial lysates. Functional evaluation demonstrated that selected postbiotic fractions exhibited significant antioxidant activity, ACE-inhibitory potential, and antimicrobial effects against model pathogens, with minimal cytotoxicity and controlled endotoxin levels. Notably, substrate type and insect source influenced both metabolic output and functional activity, highlighting the role of microbial ecology in tailoring postbiotic functionality. Collectively, this study establishes a scalable and clean-label framework for agro-waste valorisation using insect gut microbiomes and positions insect-derived postbiotics as multifunctional ingredients for applications in food systems, animal feed, and cell-culture platforms.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Detoxification of *Fusarium* toxins in black soldier fly larvae (DetoxBug)

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Black soldier fly larvae (BSFL, *Hermetia illuscens*) are of growing interest as sustainable ingredient in agri- and aquafeeds. Their capability to tolerate elevated levels of dietary contaminants can make them essential tools for the revalorisation of grain spoilt with mycotoxins. *Fusarium* spp. infection in grain is common in temperate climate and leads to harvest losses because of high contents of mycotoxins such as deoxynivalenol, T2/HT2-toxin, enniatins and zearalenone. BSFL have been shown to grow on feed containing the *Aspergillus* mycotoxin aflatoxin B1 (Heuel *et al.*, 2023); however, the exploitation of their potential for recirculating resources into the feed chain is still prohibited due to safety concerns. Thus, the established maximum levels for mycotoxins in feed are also applicable to BSFL until it has been confirmed that they do not accumulate contaminants. Studying the detoxifying abilities of BSFL with regard to *Fusarium* mycotoxins, we have exposed BSFL to chicken feed incurred with high levels of DON or T2, or to fungi-inoculated oat grains. The larvae received the contaminated feeds between days 7 and 15 of their lifecycle. BSFL mortality and growth performance were determined. Larvae and frass were collected at four timepoints, extracted and analysed with a validated multi-mycotoxin liquid-chromatography high-resolution mass spectrometry (LC-HRMS) method (Ivanova *et al.*, 2026). Larval growth remained unaffected even at substantial dietary mycotoxin levels, which were comparable to or above the highest detected levels in harvested grains. Mortality was low. The different mycotoxins were determined in feed, frass and larvae by LC-HRMS, showing that the concentrations in the larvae were low and decreasing at the different sampling timepoints. In contrast, mycotoxin levels were substantial in the frass and increasing throughout the study period. These findings indicated that the BSFL were apparently able to eliminate feed-borne mycotoxins from their organism by detoxification and excretion after they had adapted to the contaminated diet. In conclusion, the DetoxBug study showed that BSFL fed with elevated levels of different *Fusarium* mycotoxins could be safely used as protein and lipid sources in animal and fish feeds, whereas the frass is unsuitable for applications in feed. References: Heuel M., Kreuzer, M., Gangnat, I.D.M., Frossard, E., Zurbrügg, C., Egger, J., Dortmans, B., Gold, M., Mathys, A., Jaster-Keller, J., Weigel, S., Sandrock, C. and Terranova, M., 2023. Low transfer of cadmium, lead and aflatoxin B1 to eggs and meat of laying hens receiving diets with black soldier fly larvae reared on contaminated substrates. *Animal Feed Science and Technology* 304: 115733.
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INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Boosting the health properties of edible insects while valorising by-products

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Edible insects are a sustainable source of nutrients and bioactive compounds, whose health properties depend on insect species and diet. In this study, we approached that feeding insects with bioactive-rich by-products is an innovative strategy to valorise side streams while enhancing the bioactivity of insect-derived products. To illustrate this idea, we evaluated the incorporation of well-known bioactive-rich by-products, such as olive leaves (OL) and olive pomace (OP), into the diets of *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) larvae and its effect on multi-bioactive properties. Larvae were reared on wheat bran–based diets or on diets partially substituted with OL (15, 30, 50%) or OP (30, 50, 70, 90%). Reared larvae were slaughtered, dried, ground, and defatted. *In vitro* bioactivities were assessed after ultrasound-assisted extraction with 70% ethanol, including antioxidant (DPPH assay), anti-inflammatory (lipoxygenase -LOX- inhibition) and hypolipidemic (pancreatic lipase inhibition) activities, expressed as IC_{50}. Selected OL30 and OP30 samples were further tested in cellular models (RAW264 macrophages for reactive oxygen species -ROS- as antioxidant method, and for nitric oxide -NO- production as anti-inflammatory activity). *In vitro* antioxidant activity was higher for TM than HI, regardless of diet. However, TM showed no detectable antioxidant activity in cell models, while HI showed the same pattern both *in vitro* and in cells. For both species, feeding olive oil by-products increased their antioxidant capacity. HI showed a higher overall anti-inflammatory activity than TM, both by *in vitro* and cell models. The feeding of olive oil by-products enhanced this bioactivity, with the improvement being more evident for HI. Similarly, hypolipidemic activity was higher for HI than TM, with the inclusion of olive oil by-products improving such activity, more markedly for TM. These findings highlight the potential of using olive oil industry by-products into insect diets as an effective and sustainable strategy to simultaneously boost the health properties of edible insects while valorising agri-food side streams. Acknowledgment: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Grassland biomass as a substrate for black soldier fly larvae production

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In many regions in Germany, structural changes in livestock production have reduced the demand for grass feed. Consequently, grasslands are either underutilised, with biomass no longer fully harvested or economically valorised, or extensively managed, with reduced inputs, low grazing pressure, and infrequent mowing. The decline in livestock as primary biomass consumers leads to reduced management intensity and the accumulation of low-valuable biomass. These conditions favour the spread plant species, that are toxic to ruminants or monogastric animals. Alternative uses for grassland biomass are needed and rearing black soldier fly larvae (BSFL) representing a promising yet unexplored option. However, insect meal production commonly relies on substrates also suitable as feed for pigs and poultry, such as the Gainesville diet (GD). To improve sustainability and minimise competition for feed resources, alternative substrates to the GD are needed. Five-day old (DOL) BSF-larvae (initial weight of 1.07mg/larvae) were allocated equally to 7 treatments. In each case, 14 000 BSFL were housed in one of 21 identical plastic boxes (40 × 60 cm) and reared in a controlled climate chamber (28–30 °C, 55-65%RH). The experimental setup consisted of GD (50% wheat bran, 30% corn, 20% Alfalfa) as control group (CON). The following 6 treatments consisted of fresh grass, hay, and silage, 25% and 50% respectively in GD, resulting in the treatment groups Grass25, Grass50, Hay25, Hay50, Silage25 and Silage50. Grassland biomass was harvested from a single grassland site. The grass, hay, and silage were processed to the finest possible particle size to optimise their availability for the larvae. Each substrate had an initial dry matter of 20–25%. To determine BSFL growth, 50 larvae were removed from each box every 2 days and weighed to calculate the average weight per larvae and treatment. PH value and temperature of rearing substrates were measured every 2 days. Data were analysed using one-way ANOVA ($p < 0.05$), and post-hoc-Tukey HSD. After 9 days of trial, no significant differences among treatments were observed ($p > 0.05$). Individual larvae weight was highest in CON, and Silage50 (107mg/larvae), whereas the remaining treatments averaged 91-101mg/larvae, with Gras50 showing the lowest mean value (91mg/larvae). Rearing substrates containing grassland biomass had higher pH values and lower temperatures compared to CON. However, no correlation between the pH value or temperature and larvae growth performance was observed. In conclusion, the utilisation of grassland biomass in combination with GD represents a suitable substrate for BSFL rearing. Further research is needed to investigate different inclusion levels, processing strategies and larvae characteristics.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Effects of agricultural by-products and surplus food-based diets on *Hermetia illucens* larval biomass and frass quality

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Food waste is a global concern, significantly contributing to the growing challenges of waste management. The use of insects such as black soldier fly larvae (BSFL, *Hermetia illucens*), in organic waste management has emerged as a promising sustainable solution for the revalorisation of such waste through conversion to insect protein. This bioconversion process reduces waste volume and generates protein-rich larval biomass for animal nutrition and nutrient-dense frass for soil and plant nutrient supply. This study examined how different, seasonally available agricultural by-products and surplus food-based diets affect BSFL composition and frass nutrient profiles. Three diets namely: Summer (tomato, French beans, wheat bran, whey protein), Winter (potato, wheat bran, whey protein), and Control (wheat bran, layer's grain) were tested at a commercial insect farm facility over 13 days. Approximately 10 000 eight-day-old BSFL were added to 10 kg of each diet with 10 replicates per diet. Larvae fed summer and winter diets exhibited higher gross energy (24 MJ/kg) and protein content (46%) compared to the control (22 MJ/kg and 39%, respectively), likely due to the greater nitrogen and crude protein content of the respective substrates. Across diets, BSFL biomass contained 29 – 30% fat, and essential macro- and micro-elements consistent with literature. Moreover, during the first 10 days of the experiment, larvae reared on summer and winter diets were larger (summer: 1.6 cm, 126 mg/larva; winter: 1.4 cm, 91 mg/larva) than those in the control group (1.2 cm, 40 mg/larva). Frass from all treatments was rich in essential nutrients (N, P, K, Ca, Mg), with NPK ratios of 1:1.8:1.5 (Control), 1:1.9:2.4 (Summer) and 1:1.2:2.3 (Winter), and C/N ratios of 14.4 (Control), 17.4 (Summer) and 18.8 (Winter) comparable to solid manure and bio-compost, indicating suitability as an organic soil amendment. Trace elements and heavy metals (Cu, Zn, As, Cd, Pb, Ni) fell within EU regulatory limits, except for chromium (Cr) in the control diet frass, which exceeded the permissible level (3.15 mg/kg vs. the 2 mg/kg limit). These findings highlight the impact of different diets on the growth rate and nutrient content of BSFL and show that diet composition should be considered when formulating optimum diets for BSFL production systems.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Bugs in the system: have the economic and environmental benefits of insect farming been overstated?

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Insect farming has attracted over $2 billion in investment, positioned as a sustainable route to reducing dependence on fishmeal and soy in animal feed. The sector's value proposition rests on key assumptions: that insects can be fed on food waste, that production costs will decrease with scale, and that insect-based products offer environmental benefits over conventional alternatives. However, much of the supporting evidence comes from small-scale or pilot studies, often under conditions not representative of Western industrial production. This presentation examines whether these assumptions hold in light of recent developments.

This work draws on a series of peer-reviewed critical reviews by the author, synthesising life cycle assessment data, market and cost data, and industry evidence. Results indicate that insect meal currently costs $3800–6000 per ton, compared to $1400–1800 for fishmeal and approx. $500 for soybean meal, a gap that available modelling suggests is unlikely to close under realistic near-term conditions. Investment in the sector has declined sharply since 2022, and several leading companies, which collectively received over €900 million in investment, have gone bankrupt or entered insolvency proceedings, raising questions about commercial viability at scale.

From an environmental perspective, the circular economy narrative central to the sector remains largely aspirational, as large-scale farms rarely utilise genuine food waste due to regulatory, logistical, and nutritional barriers. Our review of life cycle assessments found that when food waste is not used, greenhouse gas emissions for insect meal range from 3.0 to 30.2 kg CO_2e per kg of protein. These findings are consistent with a UK government-commissioned LCA, which reported 12.9 to 30.1 kg CO_2e per kg of protein, approximately 1.8 to 13.5 times the impact of fishmeal and soybean meal, respectively. In the pet food market, the comparison is similarly unfavourable, as conventional pet foods primarily rely on low-impact slaughterhouse by-products. In the human food market, a global market audit found that only 7% of commercially available insect-based food products are marketed as meat alternatives, meaning insect-based foods mostly displace plant-based foods, which have low environmental footprints, further undermining the sustainability case for entomophagy.

These findings suggest that the gap between theoretical promises and industrial realities is substantial. For the sector to build a credible future, research and investment decisions should be grounded in evidence that reflects actual operating conditions rather than optimistic projections.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Production of black soldier flies (*Hermetia illucens*) from fish residues for sustainable aquaculture

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Aquaculture feeds are still heavily dependent on fishmeal and fish oil, contributing to overfishing and ecosystem degradation. Black soldier fly (*Hermetia illucens*) larvae offer a sustainable alternative by valorising organic waste, including fish residues, for circular economy applications. This study aimed to optimize a BSF larval rearing substrate using fermented fish waste, with larvae intended for inclusion in formulated feeds for European seabass (*Dicentrarchus labrax*). Two successive rearing cycles with the same diets were conducted (4 diets in triplicate/cycle): control 100% chicken feed and three experimental diets containing 25% fermented fish waste following the fermentation protocol of Dallaire-Lamontagne et al. (2025), blended with variable bread and vegetable waste proportions; BR25 (25% bread-50% vegetable), BR50 (50% bread-25% vegetable), BR37.5 (37.5% bread-37.5% vegetable). In each rearing cycle approximately 4 grams of newly hatched larvae (1-day) were collected and reared for 5 days in chicken feed. Plastic crates measuring 48 × 38 × 15 cm were each stocked with approximately 7000 larvae (5 days old) with an initial individual weight of (Cycle 1: 18.1 mg; Cycle 2: 11.3 mg). Larvae were fed at 0.5 g feed per larva under controlled conditions (27 °C, 60 – 70% RH), with improved temperature stability in Cycle 2. Daily monitoring of 50 larvae per crate set cycle end (<10% daily weight gain). Growth (final weight, biomass, GI), survival (SR), and bioconversion (FCR, ECI, FR) parameters were assessed. Diet composition significantly affected performance ($p < 0.05$), with Cycle 2 showing superior results. BR50 exhibited the highest performance: final weight 172.0 ± 6.1 mg, FCR = 3.52 ± 0.06, ECI = 34.24 ± 0.28%, comparable to the control (170.7 ± 1.5 mg, FCR = 3.33 ± 0.14, ECI = 37.27 ± 1.53%) and superior to other studies reporting 25 and 20% fish waste. BR37.5 showed intermediate performance, BR25 was consistently lowest (final weight 126.3 mg, FCR = 5.61). These results demonstrate that the BR50 diet can effectively replace conventional feed and supports the sustainable valorisation of organic wastes through black soldier fly larval bioconversion. Reference: Dallaire-Lamontagne, M., Lebeuf, Y., Saucier, L., Vandenberg, G.W., Lavoie, J., Allard, J.-M. and Deschamps, M.-H., 2025. Optimization of a hatchery residue fermentation process for potential recovery by black soldier fly larvae. Poultry Science 104: 104946.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Social Life Cycle Assessment of insect-based protein supply chains

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Insect-based proteins are increasingly promoted as sustainable protein alternatives to conventional protein feed ingredients. Yet, existing studies rarely assess Social Life Cycle Assessment (SLCA), predominantly evaluating the environmental or economic dimension, which limits the ability to identify sustainability across all dimensions. This study applies a reference scale following ISO 14075, prototyping the Social Hotspot Database (SHDB) to evaluate five insect-based protein production systems across different regions. Social risk scores for the insect-protein production system were calculated by converting the inventory data and were expressed in monetary terms (Medium Risk Hours and Labour Hour Risk). The Functional Unit for the study is 1 kg of insect-based protein. In total, five countries have been assessed (Italy, Greece, Germany, Tunisia, Morocco), from which only two countries/products are herewith addressed (Tunisia and Morocco) due to their significance. At the national level, Tunisia and Morocco exhibited comparatively higher composite risks, indicating weaker baseline social protection conditions. However, our results illustrate that among all products assessed, Product 3 (black soldier fly produced in Tunisia) showed a lower final social risk score than expected based on its national composite risk, whereas Product 4 (BSF Morocco) demonstrated the highest risk among all cases. Tunisia had a composite risk of 0.42 and a final social risk of 0.66, whereas Morocco had a composite risk of 0.40 but a final social risk score of 0.93. Conversely, the high final risk of Product 4 shows that favourable baseline conditions alone do not guarantee strong social performance; product-specific efficiencies within the sector can either mitigate or exacerbate social outcomes, particularly in regions characterised by higher social risk factors. The underlying social indicators in the SLCA conducted were manually extracted and represent sector-level averages; they might not capture the full heterogeneity of labour conditions between producers and the insect industry, which inherently limits granularity. Overall, the study demonstrates that sustainable insect-protein production, as assessed through SLCA, is highly dependent on product-specific characteristics along with regional conditions.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Growth, performance and larval composition of *Hermetia illucens* reared on industrially processed former foodstuffs

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Rising global populations and limited arable land require innovative, sustainable methods for food and feed production. Local insect production can be economically and environmentally sustainable if insects are reared on low-opportunity-cost substrates that would otherwise have a higher environmental impact. While current research mainly focuses on optimising black soldier fly larvae (BSFL; *Hermetia illucens*) growth using predefined diets, studies on real-life former foodstuffs are lacking. These organic streams have significant potential, as their use in animal feed can prevent their classification as organic waste, thereby avoiding the costs and environmental burdens associated with waste legislation. In this study, we tested real-world pre- and post-consumer waste-derived substrates, with and without packaging, sourced from a large-scale organic waste processor handling heterogeneous agri-food sector waste. We evaluated five diets in a standardised insect bioconversion assay with BSFL: (1) broiler starter feed (control), (2) pasteurised canteen waste, (3) retail food waste (without meat, with packaging), (4) retail food waste (without meat, without packaging) and (5) retail food waste (including meat, with packaging). We calculated biomass gain (BG), feed conversion ratio (FCR), specific growth rate (SGR), waste reduction efficiency (WRE), bioconversion efficiency (BCE), nitrogen bioconversion efficiency (NBCE) and survival rate (SR). Nondigested residues in frass were quantified, and proximate composition (Weende analysis) and heavy metal content (Mn, Zn, Cd, Pb) were analysed for both substrates and harvested BSFL. Pre-consumer expired foods with and without packaging supported growth (SGR up to 35.7% per day; BG ≈ 25% of initial larval mass) comparable to the control, with SR of 66 – 72% (control: 70%) and WRE of 68 – 71%. These substrate streams can be efficiently converted by BSFL into protein- and lipid-rich biomass with high waste reduction potential. In contrast, the post-consumer canteen waste performed poorly across all metrics, indicating that untreated kitchen waste requires structural bulking and improved aeration. Manual depackaging after grinding reduced packaging residues in frass from 6.6 to 1.8% of the initial substrate and increased NBCE from approximately 69% to 80%. Proximate analysis revealed similar BSFL nutritional profiles despite varying substrate compositions. Heavy metal content was low in all substrates and harvested BSFL, supporting compliance with EU feed safety standards when legally permitted substrate categories are used.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Impact of adding goat cheese whey to the substrate and the slaughter method on *Hermetia illucens* larvae powder quality

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The increase in global demand for sustainable food has driven interest in edible insects due to their high nutritional value and production efficiency. *Hermetia illucens* (BSF) has high potential and used in animal feed. This study aimed to evaluate the effect of supplementing the substrate with goat cheese whey (GCW) and slaughter methods on the quality of BSF larvae powders. The larvae reared on a standard chick feed based substrate, moistened with distilled water or goat cheese whey (GCW). After harvesting, the larvae killed by deep freezing or blanching, dehydrated, ground, and analysed for nutritional and microbiological quality. The powders obtained had average protein 29 ± 2.18% and ash 8.3 ± 0.6% contents, with no significant differences between diets or slaughter methods. Total fat content was 31.2 ± 2.2% with significantly higher values in larvae from the whey diet compared to the control ($p = 0.0146$, n = 12). Total fibre content was 9.5 ± 0.9%, being significantly higher in the whey diet ($p < 0.001$, n = 12). The mesophilic aerobic count ranged from 3.43 to 6.08 log CFU/g, with half of the samples exceeding the legal limit (≤ 10^5 CFU/g). In the mould (<1–3.54 log CFU/g) and yeast (<1–3.97 log CFU/g) counts, half of the samples exceeded the legal limit (≤100 CFU/g). Related to sulphite-reducing anaerobes and *B. cereus*, 4 samples exceeded legal limits. Non-conformities are possibly associated with fungal contamination of the substrate supplied to the larvae or recontamination of the final product. Pathogens and indicators of faecal contamination were within legal limits in all samples. Supplementing the substrate with GCW improved the nutritional profile of BSF, increasing fat and fibre content without affecting protein and mineral content. Slaughter methods did not significantly influence microbiological quality. Acknowledgements: Work carried out within the scope of the project InsectERA (C644917393-00000032), funded by the Recovery and Resilience Plan (RRP), Portuguese Republic, Measure RE-C05 — Mobilizing Agendas for Business Innovation and European Union, NextGenerationEU.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Olive pomace application of feedstock for *Hermetia illucens* after different chemical and thermal pretreatments

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Spain is the world's largest producer of olive pomace (OP). Olive pomace is a semi-solid by-product of the olive oil industry composed of pulp, skin, olive pit fragments, and water. It presents significant environmental challenges due to its complex chemical composition. Among the alternative strategies proposed for olive pomace valorisation, its use as feedstock for black soldier fly larvae (BSFL, *Hermetia illucens*) has emerged as a promising option. However, OP's high lignin content, organic load, and phytotoxicity make its direct use for BSFL rearing challenging. This study evaluated the impact of different pretreatments on olive pomace properties and its suitability as feedstock for BSFL. Acidic pretreatments using hydrochloric acid (1M), hydrothermal pretreatments at 160, 175 and 190 °C, and thermal pretreatments at 28, 40, 50, 60, 70 and 80 °C were applied to the olive pomace. Changes in reducing sugar and polyphenol content in OP following pretreatment were monitored. The efficacy of each pretreatment was assessed through BSFL growth trials, with performance compared to larvae reared on chicken feed and non-treated olive pomace. Acid pretreatments removed more polyphenols (>200 ppm) than hydrothermal pretreatments (19–30 ppm). Soft thermal pretreatments did not significantly affect polyphenol or reducing sugar availability. Larvae fed with olive pomace non-pretreated or pretreated with hydrochloric acid pretreatment showed similar values of biomass (0.013 g dw/larva). This was significantly lower than the larvae reared on chicken feed (0.070 g dw/larva). Hydrothermal pretreatments also showed low yields with larva biomass values of 0.012, 0.007 and 0.001 g dw/larva at 160, 175 and 190 °C. Finally, soft thermal treatments also showed limited effectiveness, with BSFL biomass across all temperatures reaching approximately 0.015–0.011 g dw/larva). These preliminary tests showed that the tested acidic and hydrothermal pretreatments conditions removed polyphenols and reducing sugars. However, these effects were not translated in higher BSFL growth. This, combined with high environmental risks and costs, suggests that future pretreatment research should focus on alternative approaches such as basic pretreatments or co-digestion strategies.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Trade-offs in insect-based waste valorisation of spent coffee

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Global coffee consumption generates a large stream of organic waste that could be valorised through insect-based bioconversion. This study examined how incorporating spent coffee grounds (SCG) into a high-quality black soldier fly (BSF, *Hermetia illucens*) diet affects insect performance and the fertilization potential of the resulting frass. BSF larvae were reared on a Gainesville diet supplemented with increasing proportions of SCG (0, 10, 25 and 50%), and larval growth dynamics, development time, and adult traits were quantified. Subsequently, basil (*Ocimum basilicum*) and duckweed (*Lemna minor*) were grown in substrates amended with frass derived from selected diets (0, 25 and 50% SCG) at varying application rates (0, 10 and 20% for basil and 0, 10 and 20 g/l for duckweed). Increasing SCG content reduced larval growth rate, peak weight and life history traits, indicating costs to insect performance. By contrast, frass derived from coffee-supplemented diets enhanced plant growth in both plants when applied at moderate rates (10% for basil or 10 g/l for duckweed). These results reveal a trade-off between BSF performance and frass quality and highlight the potential of BSF bioconversion to transform spent coffee grounds into value-added agricultural inputs when application rates are optimised.

INSECTS IN WASTE MANAGEMENT AND SUSTAINABILITY

Effects of feeding on *Salix gordejevii* on the growth and development of *Protaetia brevitarsis* larvae

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As a saprophagous resource insect, *Protaetia brevitarsis* larvae have the potential to efficiently transform organic agricultural and forestry wastes. With high protein content and abundant amino acid composition, the larvae are high-quality animal protein sources. *Salix gordejevii* is one of the key tree species for windbreak and sand fixation in the sandy areas of northern China. Due to its high fibre content, poor palatability, and rich secondary metabolites such as phenols, its utilisation methods are relatively single, mostly used for sand barriers and weaving, with insufficient resource utilisation. To efficiently utilise *Salix gordejevii* resources, fermented *Salix gordejevii* feed was prepared by adding lactic acid bacteria and *Bacillus subtilis* to *Salix gordejevii* in this study. A feeding experiment on *Protaetia brevitarsis* larvae was conducted with commercial insect feed as the blank control. The results showed that *Protaetia brevitarsis* larvae could efficiently transform *Salix gordejevii* waste, with the highest feed utilisation rate of 64.64% and larval conversion rate of 89.03%, which were not significantly different from those of the commercial insect feed ($P > 0.05$). The protein content of larvae fed with fermented *Salix gordejevii* was above 50%, with the highest value of 57.97%, and the fat content of each treatment group ranged from 2% to 4%, indicating that they are high-protein and low-fat protein sources. This study provides theoretical guidance for the resource utilisation of *Salix gordejevii* branches, an agricultural and forestry waste, and also a reference for new-type feed for the low-cost large-scale breeding of *Protaetia brevitarsis* larvae. Funding: Guizhou Provincial Major Scientific and Technological Program (2025) 004.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutritional, antioxidant and sensory acceptability in wheat biscuits fortified with black soldier fly and mopane worm

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Edible insect flours represent a promising strategy for enhancing the nutritional quality of cereal-based snacks, although the inclusion level and insect species may impact product quality and consumer acceptance. This study evaluated the effects of fortifying wheat biscuits with flours from *Gonimbrasia belina* and *Hermetia illucens* on their nutritional composition, antioxidant capacity, and sensory acceptability. Biscuits were produced by replacing wheat flour with *H. illucens* or *G. belina* flour at 5, 10, 15 and 20% (w/w). Proximate composition, colour parameters, antioxidant indices including total phenolic content, DPPH, and ABTS, and sensory acceptability were determined. Protein content increased significantly with insect inclusion compared to the control ($p < 0.05$), ranging from 14.09 to 17.80% for *H. illucens* and 15.04 to 19.91% for *G. belina*. The fat content was significantly higher in *H. illucens* biscuits compared to the control ($p < 0.05$), while differences among inclusion levels of 5 to 15% were not significant ($p > 0.05$). Moisture and ash contents increased significantly in all insect-fortified biscuits ($p < 0.05$). Antioxidant capacity increased significantly with inclusion level ($p < 0.05$), with total phenolic content rising from 6.05 mg GAE per 100 g in the control to 251.14 mg GAE per 100 g for *H. illucens* and 77.43 mg GAE per 100 g for *G. belina*. Sensory acceptability declined significantly at higher substitution levels ($p < 0.05$). PCA separated low and high inclusion treatments, with PC1 and PC2 explaining 45.93 and 23.04% of total variance. Insect flour fortification significantly enhanced nutritional and antioxidant properties, while sensory constraints limited acceptable inclusion levels. Lower substitution levels represent the most viable balance between functionality and consumer acceptance

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutritional quality of edible insects: impact of species, developmental stage, sex and fermentation

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As the insect farming industry grows, understanding how species, developmental stage, sex, and processing methods affect nutritional composition is crucial, as variations in fat, protein, carbohydrate and mineral content arise from these factors. Additionally, fermentation, a traditional processing method, can enhance sensory qualities, improve nutrient bioavailability, reduce anti-nutritional factors, increase bioactive compounds, support gut health and extend shelf life. This study assessed how species, developmental stage, sex and fermentation influence the nutritional quality of farmed insects. Seven species (desert locust, migratory locust, yellow mealworm, house cricket, Jamaican field cricket, lesser mealworm and discoid cockroach) of different sex and developmental stage were analysed for nitrogen, fatty and amino acids, elements and polyphenols. After that, yellow mealworm, house cricket and migratory locust were fermented with *Lactobacillus plantarum* and a commercial starter culture for 48 hours and then analysed for proximate composition, fatty and amino acids, elements and oxidation stability. Results showed that species, developmental stage, sex, and fermentation significantly affected nutritional quality. Insects were rich in essential fatty acids, amino acids, and minerals. Polyphenol content ranged from 7.6 to 511 mg/kg dry weight, with the highest levels in female desert locust (511 mg/kg, 19 compounds), adult migratory locust (288 mg/kg, 34 compounds), and discoid cockroaches (male 449 mg/kg, 21 compounds; female 357 mg/kg, 20 compounds). Fermentation reduced dietary fibre in yellow mealworm (33%) and house cricket (12%) but increased non-protein nitrogen by 38% and 16%, respectively. Fatty acid profiles were largely unchanged, while amino acid composition varied by species and fermentation culture. Mineral concentrations (Fe, K, Mg, Mn, Na, P) increased, whereas Se and Cu decreased. Zinc levels showed inconsistent trends. Oxidative stability decreased in yellow mealworm (42%) and migratory locust (25%), but improved in house cricket (160%). Overall, fermentation enhanced insect nutritional value, with variation by species and fermentation culture.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Comparative analysis of amino acids, minerals, thermal decomposition, and colour in six full-fat edible insect species

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Understanding species-level variability in edible insects is fundamental for their use as food ingredients. However, comparative datasets integrating protein composition, micronutrient content, thermal behaviour and colour across multiple insect species remain limited. Six edible insect species, *T. obscura*, *T. molitor*, *H. illucens*, *G. bimaculatus*, *R. differens* and *S. gregaria*, were analysed in the full-fat state using triplicate samples. Amino acid composition, mineral content, thermal stability, and colour attributes were quantified and evaluated using multivariate analysis of variance (MANOVA) to determine overall species effects. Amino acid profiles differed significantly among species ($p < 0.001$), with clear separation observed for total, essential, hydrophobic, and charged amino acid fractions. Mineral composition was likewise species dependent ($p < 0.001$), revealing distinct macro- and trace-mineral signatures across species. Thermogravimetric analysis further demonstrated species-specific thermal decomposition behaviour, indicating differences in thermal stability and compositional architecture among insects. Colour attributes also varied markedly, with S. *gregaria* exhibiting higher lightness and chroma, while *H. illucens* and *T. molitor* displayed darker, less saturated profiles. Within-species variability was low across all measurements. The pronounced inter-species differences observed across nutritional composition, thermal behaviour, and colour attributes demonstrate that edible insects represent a diverse ingredient space rather than a uniform protein category. From an industrial perspective, these baseline differences have direct implications for ingredient specification, formulation strategy, nutritional positioning, and consumer acceptance.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Multivariate evaluation of nutritional, techno-functional, and structural properties of wheat-mopane worm composites

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Edible insects are increasingly recognised as sustainable protein sources, yet their interaction with cereal matrices during processing remains poorly understood. This study investigated how mopane worm (*Gonimbrasia belina*) powder influences structure–function relationships in wheat-based composite flours. Methods Composite flours were produced by substituting wheat flour with mopane worm powder at 10, 20 and 30% (w/w). Nutritional composition, techno-functional, pasting behaviour, thermal stability, and molecular structure were characterised using standard analytical techniques, including Rapid Visco Analysis, thermogravimetric analysis, and FTIR spectroscopy. Results Mopane worm inclusion significantly increased protein and mineral content while reducing carbohydrate proportion ($p < 0.05$). Hydration behaviour was altered, with 100% mopane powder exhibiting a significantly higher water binding capacity than wheat flour, while composite blends showed moderated responses. Pasting profiles were markedly affected, with peak viscosity decreasing from approximately 1920 cP in wheat flour to below 900 cP at 30% inclusion, alongside significant reductions in breakdown and setback viscosities. Thermal analysis showed delayed degradation in insect-enriched flours, indicating improved thermal resilience. FT-IR spectra demonstrated progressive intensification of amide I and II bands and aliphatic stretching vibrations, confirming a transition from starch-dominated to protein-dominated matrices. PCA explained over 80% of the total variance and revealed distinct clustering driven by protein content, colour metrics, and functional properties. Discussion and conclusion Mopane worm powder induces coordinated structural and functional transitions within wheat flour systems. These findings provide mechanistic insight into insect–cereal interactions and support the rational design of protein-enriched composite flours for processed food applications.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Protein/peptide bioaccessibility and bioactivity of five traditional edible insects from Mexico after INFOGEST digestion

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Edible insects are increasingly recognized as sustainable sources of high-quality nutrients and bioactive compounds. This study evaluated the protein digestibility, nutritional quality, and antioxidant capacity of five traditionally consumed edible insect species from Mexico: mesquite bug (*Thasus gygas*), maguey white worm (*Aegiale hesperiaris*), maguey red worm (*Comadia redtenbacheri*), milpa grasshopper (*Sphenarium purpurascens*) and leafcutter ant (*Atta mexicana*). Samples were subjected to standardized *in vitro* gastrointestinal digestion following the INFOGEST 2.0 protocol. Protein solubility, soluble peptide content, amino acid profile, mineral composition, *in vitro* antioxidant activity (ABTS, reducing power, iron, and copper chelation), cellular antioxidant activity (CAA), and reactive oxygen species (ROS) inhibition in Caco-2 cells were assessed before and after digestion. Protein solubility increased between 3- and 12-fold ($p < 0.05$) depending on the species, with *T. gygas* showing the highest increase. Soluble peptide content increased significantly after digestion, reaching up to 70-fold ($p < 0.05$) in some species (*C. redtenbacheri* and *A. mexicana*), although the highest absolute peptide concentrations were observed in other insects (*A. hesperiaris* and *T. gygas*). *In vitro* antioxidant assays showed markedly enhanced activity after digestion, with values up to four times higher than undigested samples ($p < 0.05$). CAA increased by 14.23–43.67% ($p < 0.05$) compared to untreated Caco-2 cells, and ROS inhibition reached up to 85.26% ($p < 0.05$) in digested *T. gygas* (after intestinal phase 120 min). Amino acid profiles indicated enhanced protein quality in all five species, even before digestion (≥1.0 EAAI, ≥100 AAS, ≥100% BV). Additionally, mineral profiling revealed a wide diversity of macro- and microelements across all species, while heavy metals and medically relevant elements remained below hazardous levels. These findings demonstrate that gastrointestinal digestion substantially improves the bioaccessibility and bioactivity of proteins and antioxidant compounds from traditional Mexican edible insects. The high nutritional quality and improved antioxidant effects support their potential as functional and sustainable food ingredients for human nutrition.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Structure and gelling properties of house cricket (*Acheta domesticus*) myofibrillar proteins

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Incorporation of edible insect meals into meat formulations has been reported to negatively affect texture and colour at inclusion levels above 10% (w/w), thereby limiting their use as meat alternatives. The textural properties of meat products are largely governed by the gelling behavior of myosin. House crickets (*A. domesticus*) contain a high proportion of muscle proteins, including myosin. Thus, this study investigates the structural characteristics and gelling properties of isolated cricket myofibrillar proteins. Crickets (*A. domesticus*) were killed by flash-freezing and subsequently freeze-dried. Myofibrillar proteins were extracted by ammonium sulfate precipitation (50% saturation), followed by dialysis and desalting by ultracentrifugation. Protein profile was analysed by SDS-PAGE, while protein structural properties were characterised through surface hydrophobicity (H_0), free and total sulfhydryl groups and differential scanning calorimetry. Modifications in protein secondary structures were visualized by FTIR. Protein suspensions (1–5% (w/v) proteins, 0.6 M KCl, pH 7.0) were heated at 80 °C for 1 h to determine the least gelation concentration and water holding capacity. Gel strength was assessed by rheological measurements. Protein interactions involved in gel network formation were investigated using destabilising agents (1 M urea, 8 M urea and 0.1 M DTT) combined with Bradford method and SDS-PAGE analysis. Protein concentration of the myofibrillar protein-enriched extract was 63 ± 2%, with myosin heavy chain (25%), paramyosin (16%) and actin (17%) as the predominant proteins. Cricket myofibrillar proteins formed gels at 25 °C and 80 °C at a minimum concentration 3% *m*/*v*, exhibiting a water holding capacity of 98%. Differential scanning calorimetry revealed two denaturation peaks at 51 °C and 83 °C. H_0 increased markedly at 70 °C, whereas free sulfhydryl group content decreased at temperature exceeding 50 °C. Rheological analysis showed that the solid-like behaviour of a 3% protein suspension observed at 25 °C was thermo-reversible above 50 °C. Gelation initiated at 65 °C and strengthened upon cooling, resulting in a weak gel (G' = 25 Pa) with characteristics more similar to polysaccharide gels. SDS-PAGE analyses suggest that myofibrillar gel network formation is predominantly formed through hydrophobic interactions. Cricket myofibrillar proteins exhibited structural characteristics comparable to those of animal muscle proteins but formed weaker gels. Further work should investigate cricket-animal myofibrillar protein systems to better model the substitution of animal-derived ingredients by insect-based alternatives in processed meat products.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Characterisation and application of *Acheta domesticus* in sustainable food systems

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This work presents an integrated framework encompassing farming, processing, and formulation of cricket (*Acheta domesticus* L.) ingredients to support sustainable food systems. First, diet optimisation through cricket self-selection of regional agro-industrial by-products under an industrial farming system was evaluated. This was followed by an assessment of how commercial processing conditions affect the nutritional composition and techno-functional properties of edible crickets. Subsequently, the protein profile and techno-functional potential of cricket protein concentrates were characterised, concluding with an evaluation of insect-plant protein blending using a machine-learning approach. In the farming stage, stage-specific self-selection diets incorporating 13 Mexican agro-industrial by-products improved biomass yield (+59%), survival (+35%), and feed conversion efficiency (+14%) compared to conventional broiler feed. Ingredient preferences varied across developmental stages, with fibre-rich corn bran favoured during initiation, carbohydrate-rich corn flour during growth, and lipid-dense toasted sesame paste during reproduction, highlighting dynamic nutritional requirements. Regarding processing, the techno-functional properties and composition of different cricket fractions revealed that underutilised materials, particularly legs, may represent valuable food ingredients. Legs, head-and-torso, and whole-body crickets were systematically evaluated. Legs exhibited the highest protein content (75.2 g/100 g dw) and distinct cuticular protein bands (approx. 20 kDa, <15 kDa). Protein digestibility was highest in head-and-torso fractions (85.5%, PDCAAS = 0.86) and lowest in legs (PDCAAS = 0.73). ATR-FTIR analysis indicated predominant β-sheet structures, especially in legs (89.6%), suggesting a higher degree of protein aggregation. Finally, ingredient application was explored using a dataset of 124 formulations (66 single ingredients and 58 binary blends spanning insect-insect, insect-plant, and plant-plant combinations). Machine-learning models linked compositional inputs to five key techno-functional outputs. Spline regression performed best overall (R^2 = 0.763, MAE ≈ 0.23 for multi-output prediction), with strong performance in oil-in-water emulsifying capacity and oil-holding capacity predictions. Overall, this work demonstrates an integrated strategy to enhance crickets as a versatile source of food ingredients by addressing bottlenecks in production, processing, and formulation. Together, these findings provide a foundation for expanding the application of cricket-based ingredients in sustainable food systems.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

The edible insects of Tanzania: current potential and future opportunities

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Edible insects are considered the fastest-growing alternative protein market in the world. The practice of eating insects by humans is known as human entomophagy, and people throughout the world have been eating edible insects as a regular part of their diets for millennia. It is considered safe as there are no reported cases of disease transmission, parasitoids, or heavy metals contamination to humans from the consumption of insects. The field of Entomophagy is under- researched in Tanzania, and there are no specific policy issues for it. Despite the fact that edible insects are reported to contain more nutrients like protein, fats, vitamins and minerals of high value compared to other conventional sources, bioactive compounds, and medicinal traits, there is limited data. The current study is looking at the occurrence, habitats, indigenous processing methods, cultures, beliefs, nutritional composition and value addition of the edible insects of Tanzania. This is being done through ethnography, key informant interviews, observations, literature, the internet, chronological reports and experimental laboratory analysis. Several tribes in Tanzania are reported to have edible insects as part of their traditional diets with cultural significance, and some treat them as respectable delicacies. Commercialisation and consumption of processed edible insects such as longhorn grasshopper (senene) is increasing countrywide. There are several farms identified in Tanzania that are practising insect rearing primarily for animal feed. There are different value-addition possibilities with scaling up potential, focusing on nutrition improvement in traditional diets and livelihood improvement in low-income societies. Innovations in the value addition of edible insects have the potential to fight malnutrition and poverty following increased commercialisation. Regulatory bodies and policy frameworks need to include the adoption of edible insect aspects, like food safety standards covering production to consumption.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutritional characterisation of 30 insect species for their potential as food or feed

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The rising global demand for sustainable protein sources has intensified interest in alternative food and feed systems, with edible insects gaining increasing attention. We evaluated 30 insect species from Nepal for their nutritional potential as future food or feed. Species identification was initially based on morphological traits and subsequently confirmed using DNA barcoding. After sample collection, they were freeze-dried, ground, and analysed for proximate composition, amino acid, and fatty acid profiles. Preliminary results revealed substantial interspecific variation in nutritional composition. Crude protein content ranged from 30 to 60%, with orthopterans exhibiting the highest levels. Mulberry silkworms, scorpions, wasps and water beetles also exceeded 50% crude protein. Crude fat content varied widely (5–45%). Amino acid profiles were generally balanced and met recommendations for children and adults, as well as requirements for poultry and pigs, except for methionine. Among insect species, scavenger species, including mole crickets, black soldier flies, blowflies and certain lepidopteran larvae, were especially rich in minerals. Overall, multiple insect species exhibit strong nutritional potential, with high-quality protein, balanced amino acids, and valuable lipid and mineral profiles, underscoring their suitability as future food and feed resources. Strategic identification, domestication, and management of locally available nutrient-rich species can substantially enhance sustainable protein supply and strengthen global food and feed security.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutritional and physicochemical characterisation of *Tenebrio molitor* and *Hermetia illucens* larvae and flours

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Global food security and sustainability challenges have driven interest in edible insects as low-impact protein sources. In particular, black soldier fly (*Hermetia illucens*, BSFL) and yellow mealworms (*Tenebrio molitor*, TNL) larvae stand out for their rich nutritional and functional properties. This study aimed to comparatively assess the nutritional composition, structural features, and techno-functional properties of BSFL and TNL, alongside their industrially processed flours (BSFF and TNF). Significant differences ($P < 0.05$) were observed among the powders in terms of composition, and physico-chemical and thermal properties in comparison with their larvae. For instance, the total lipid content decreased by 62.28 and 59.50% db in BSFF and TNF, measuring 14.56 and 10.57% db, respectively, while BSFL and TNL showed higher values of 38.60 and 26.10% DW. In contrast, the total protein content increased by 68 and 22% db in BSFF and TNF, reaching 60.84 and 72.08% db, respectively, compared with BSFL and TNL, yielding values of 36.22 and 59.04% db. Thermal gravimetry and differential scanning calorimeter analysis, highlight the conformational physical changes induced by the partial removal of fats which has an influence on protein denaturation. Fourier transform infrared spectroscopy further revealed that the industrial processing induced structural modifications on the amide I and amide II (1500-1700 cm^{-1}) regions. At the powder scale, morpho-granulometric analysis revealed reduced and more homogeneous particle size distributions in BSFF and TNF than in BSFL and TNL, reflecting the strong impact of industrial processing on powder structure and surface properties. These compositional and structural modifications collectively contributed to the enhanced techno-functional performance of the flours. Accordingly, BSFF and TNF showed superior techno-functional properties, with highest oil (increased by 22 and 31,4% reaching 3.33 and 3.47 g/g wb) and water holding capacity (increased by 17.7 and 11.2% reaching 4.58 and 3.47 g/g wb) than their corresponding larvae (BSFL and TNL). In addition, the mineral composition further supported the nutritional relevance of the flours. Overall, industrial processing had a different impact on BSFL and TNL, but in the two cases, it increased protein content and improved techno-functional properties, supporting insect flours as nutrient-dense ingredients for sustainable food formulations.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Molecular characterisation and assessment of potential allergens in *Hermetia illucens*

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According to the UN Food and Agriculture Organization (FAO), insects have promising potential as an alternative food source, with both environmental and health benefits. In parallel with development of regulations surrounding the use of insects for human consumption, the associated risks of food allergies should be explored. Allergic reactions to edible insects have been reported, as well as the possibility of cross-reactivity with crustaceans and house dust mites. Tropomyosin (TPM) and Arginine Kinase (AK) are, indeed, the primary allergenic proteins in crustaceans and house dust mites. *Hermetia illucens*, black soldier fly (BSF), is one of the most interesting insects for use in the human diet and, in this perspective, it is essential to assess possible cross-reactivity with known Arthropoda allergens. In this work, we investigated the allergenic potential of TPM and AK identified in BSF genome by bioinformatic analysis. Both proteins were expressed as recombinant proteins in *E. coli*. They were fully characterised by LC-MS analysis and CD spectroscopy. Bioinformatics tools were used to predict possible linear and conformational epitopes, and *in vitro* simulated digestion associated to high-resolution mass-spectrometry allowed the identification of peptides resistant to gastro-intestinal conditions, which were compared with epitopes of Arthropoda and Mollusca allergens to predict the persistence of allergenicity upon digestion. In the context of food allergy, sequential IgE binding epitopes are particularly relevant as conformational epitopes are easily degraded in the gastrointestinal tract. The identified sequences were synthesized using solid phase peptide synthesis. Preliminary NMR Conformational analyses were performed, with the aim to assess the correspondence between the epitope's solution conformation and the bioinformatically predicted structure. Finally, IgE-immunoblotting assays conducted with sera from patients with food allergies to shrimps and/or airway allergy to mites showed reactivity to both recombinant proteins and synthesized epitopes, suggesting a cross-reactivity of both *H. illucens* TPM and AK with shrimps and mites. These results highlight that the allergenicity assessment of Novel Foods is crucial to correctly inform the potentially allergic consumer. The availability of recombinant TPM from BSF will also allow the inclusion of these allergens in molecular screening aimed to specifically identify reacting allergenic molecules in view of the use of this insect and its derivatives in the human diet.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Development of corn-based extruded snack enriched with lesser mealworm flour

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The use of insect-derived ingredients in extruded snack products offers a versatile way to provide sustainable, safe, and nutritious insect-based alternatives that consumers are willing to accept. This study involved the production of corn extruded snacks enriched with lesser mealworm (*Alphitobius diaperinus*) flour, as well as the physicochemical (moisture content, water activity, water absorption index (WAI), water solubility index (WSI), swelling index, expansion index, hygroscopicity, colour) and microbiological (anaerobic mesophiles, enterobacteria, yeast and moulds) characterisation of the final products. Corn flour was partially substituted with lesser mealworm flour at varying levels: 2.5, 5, 7.5, 10 and 12.5%. The extrusion process utilised a single-screw extruder operating at 150 rpm, with a screw length-to-diameter ratio of 25:1, a compression ratio of 3:1, a die diameter of 3 mm, and a feed rate of approximately 1.58 kg/h. A control sample consisting of 100% corn flour was also included in the study. A significant reduction in moisture content was observed, decreasing from 6.54 to 5.24 g/100 g, along with a decrease in water activity from 0.450 to 0.369 as the levels of insect flour increased. All samples remained within the low-moisture range. The WAI increased (4.56–5.59 g/g), while the WSI decreased (16.81–5.35%), accompanied by reduced swelling and expansion indices, reflecting denser, less expanded structures at higher levels of insect flour incorporation. Protein content increased at all levels of replacement, peaking at 12.47 g/100 g at the highest substitution level, compared with 6.10 g/100 g in the control. At the same time, colour darkening (L* decreased from 73.93 to 65.3) and reduced chroma (29.92 to 26.0). These changes were attributed to protein-induced browning reactions during extrusion. Texture measurements demonstrated reduced hardness and crispness in samples with higher substitution levels, aligning with limited expansion and altered starch–protein interactions. Microbiological analysis showed no detectable growth in any culture media, confirming that all extruded products were microbiologically safe for consumption. Overall, lesser mealworm flour can be successfully incorporated into corn-based extruded snacks without compromising physicochemical stability or microbiological safety, with moderate inclusion levels (≤7.5%) providing optimal protein content and structural properties.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Bioassay-guided evaluation of chitin–protein fractions from *Hermetia illucens* as inhibitors of AGEs

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Advanced glycation endproducts (AGEs) are implicated in the development of chronic metabolic and age-related diseases, and there is growing interest in identifying natural inhibitors of AGE formation from sustainable bioresources. *Hermetia illucens* larvae represent a promising source of functional biopolymers, particularly chitin-based fractions, with potential health-related applications. This study investigated the antiglycation potential of fractions derived from *H. illucens* larvae using a bioassay-guided fractionation approach. Chitin–protein mix, chitin-rich fractions with minerals, chitin-rich fractions without minerals, and fat fractions were evaluated for their inhibitory activity against protein-bound fluorescent AGEs. Larvae were reared on fruit and vegetable substrates at 50 and 85% inclusion levels, as well as on food industry by-products consisting of fruits and vegetables, olive pomace, and grape marc. For fruit and vegetable substrates, fractions derived from larvae fed 85% FV showed a decreasing trend in IC_{50} values compared to 50% FV; however, these differences were not statistically significant. In larvae reared on food industry substrates, the chitin–protein mix exhibited IC_{50} values ranging from 106.96 to 210.77 μg/ml, with no significant differences among substrate types. Notably, the chitin-rich fraction without minerals derived from grape marc-fed larvae (282.40 ± 50.74 μg/ml) showed a significantly lower IC_{50} value compared to those obtained from fruits and vegetables (435.93 ± 24.70 μg/ml) and olive pomace (464.75 ± 56.84 μg/ml). Multiple comparison analysis indicated that chitin-rich fractions without minerals were consistently the least effective inhibitors compared with chitin–protein mix and chitin-rich fractions containing minerals, irrespective of substrate. Fraction type was the primary determinant of antiglycation activity, explaining over 80% ($p < 0.0001$) of the variance for fruit and vegetable substrates and nearly 69% ($p < 0.0001$) for food industry substrates, while substrate type had little to no effect. Principal component analysis supported these findings, with the first two components explaining more than 80% of total variance. Overall, the results demonstrate that inhibition of protein-bound fluorescent AGEs is driven primarily by the fraction derived from *H. illucens* rather than the rearing substrate. Chitin–protein mix and chitin-rich fractions with minerals show promise as natural AGE inhibitors; however, further studies are needed to elucidate the synergistic physicochemical properties underlying chitin's enhanced antiglycation activity.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

The effect of yellow mealworm-enriched maize porridge on growth of school-going children in Western Uganda

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Children living in food-insecure environments consume diets that are nutritionally inadequate, characterised by limited intake of animal-sourced foods (ASFs). Malnutrition in school-aged children adversely affects their physical growth, cognitive development, and academic performance. Increased consumption of ASF's can improve children's growth, nutrition and health. Edible insects are nutrient-dense and can be produced sustainably as a novel protein, alternative to conventional ASF. However, their impact on children's nutrition and health is not well established. This study investigated the effects of a yellow mealworm-enriched porridge on growth of 6–9-year-old school children living in and around Kyaka II refugee settlement in Uganda. A randomised, double blinded, two-arm parallel trial was conducted. Children were individually randomised to receive either yellow mealworm enriched maize porridge (YMP) or standard maize porridge (MP) for 14 weeks. The primary outcome was height gain and secondary outcomes were other anthropometric measurements. Dietary data was assessed by 24-hour recall. The intervention involved inclusion of 25 g/100 g (dry weight) yellow mealworm powder. A portion of 400 ml of porridge was served daily, providing 15.8 g of protein in the YMP group and 5.7 g in the MP group. Children's household diet was low in protein. Consumption of YMP significantly increased protein energy%. The YMP group showed greater height gain and lower accumulation of subcutaneous fat mass compared to the MP group. Changes in weight remained similar between both groups. Detailed results on the effect of the intervention on growth will be presented at the conference. The consumption of YMP improved linear growth in school going children with low habitual consumption of ASF's. The growth effects elicited by the yellow mealworm in this study show similarities to those associated with dairy. Therefore, the yellow mealworm may serve as an alternative and sustainable protein source for children with limited access to ASF's. This study highlights the nutritional potential of edible insects in the diets of children.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

School children acceptance of ready-to-cook pottages fortified with African palm weevil larvae as school lunch in Ghana

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This pilot study investigated nutritional content, acceptability and cost of ready-to-cook (RTC) pottages fortified with African palm weevil larvae (APW). It aimed to provide evidence as a potential public health nutrition intervention strategy in Ghana's school feeding programme (GSFP). The RTC pottages were produced from white yam or cocoyam or sweet potato and APW flours. Mixture design was used to generate 5 composites. The 5 composites obtained were processed into ready-to-cook pottages for each root and tuber crop. 50 school children aged 8 to 12 years served as sensory evaluation panellists for each root and tuber crop pottage. A modified WHO One Health Tool ingredients-based approach of intervention costing was used in estimating the cost of serving per child. Protein, fat, iron and zinc content increased with the increase in APW content of the composite. Sensory evaluation of the products showed no significant differences ($p < 0.05$) in terms of taste and overall acceptability of the RTC products. The products were highly acceptable to the panellists. 68, 84 and 92% of the panellists were willing to consume the RTC cocoyam, white yam and sweet potato pottages respectively as school lunch. The cost ranged GH₵7.33 to GH₵ 8.15 per 150 g serving for a child. The cost estimation provides a higher cost comparable to GH₵2.00, which is the current GSFP government approved budget of food per child. However, policymakers would be able to lower the improved meals cost by making available less expensive APW production and processing technologies to farmers and processors.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Edible cricket supplementation and nutrition education modulate gut microbiota of infants aged 6–14 months in Kenya

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Edible crickets can potentially replace common animal-source proteins in complementary food (CF); however, their effects on the emerging gut microbiome are not fully described. We analysed secondary output measures from a 2 × 2 factorial randomised control trial (clinicaltrials.gov: NCT06002620) to evaluate the effects of edible cricket-enriched CF on the gut microbiome based on Cricket treatment (+/–CR) and Nutrition Education treatment (+/–ED). Eligible mother-infant dyads with infants aged ≥ 6 months were enrolled at Rwambwa Sub-County Hospital in Siaya County, Kenya. Participants were randomised into treatment arms, received intervention foods +/–CR monthly as take-home rations, and were offered monthly +ED sessions delivered as audiovisual content with education messages. The +CR rations were age-adjusted (29–80 g/day) and contained 24% cricket powder. Stool samples collected at baseline and after eight-month intervention were characterised using 16S rRNA gene sequencing. Statistically significant changes in microbial diversity were observed post-intervention (Shannon $p = 0.016$; Chao1 and Observed species $p = 0.27$; Simpson $p = 0.12$). Cricket intervention increased *Faecalibacterium prausnitzii*, *Bacteroides uniformis*, *Lactobacillus reuteri* and *Akkermansia muciniphila*, which are considered beneficial taxa, and decreased potential pathogens *Escherichia coli* and *Clostridium innocuum*. The CR + ED treatment resulted in microbiome modification with significant increases in short-chain fatty acid producing species (*F. prausnitzii*, *Roseburia intestinalis*, *Coprococcus eutactus* and *Bifidobacterium adolescentis*), as well as inhibition of *Escherichia coli* and *Clostridium difficile*. These findings suggest that edible cricket supplementation can beneficially modulate the gut microbiome of infants. The additional impact of caregiver nutrition education points to complex pathways that warrant further investigation. Integrating insect-based complementary foods represents a safe and biologically meaningful strategy for supporting early-life gut health and improving child nutrition in resource-limited settings.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nixtamalization of *Sphenarium purpurascens* and its application in maize tortillas

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The growing demand for sustainable protein sources has increased interest in edible insects; however, most studies focus on thermal or fermentation treatments, while alkaline processing remains poorly explored. This study evaluated the application of nixtamalization (alkaline treatment) to adult *Sphenarium purpurascens* (SP) and its incorporation into nixtamalized maize tortillas. Grasshoppers were cooked in 1% $Ca(OH)_2$ at 98–100 °C for 0–40 min and steeped for 14 h. Physicochemical properties, protein digestibility, chitin content, calcium uptake, and protein structural changes (ATR-FTIR) were evaluated. Tortillas were produced using a 90:10 maize nixtamal:SP ratio and characterised for colour, texture, proximate composition, *in vitro* protein digestibility, and PDCAAS. Alkaline treatment of SP increased dry matter loss from 25.6% at 0 min to 31.5% after 40 min of cooking, accompanied by a pH increase from 4.8 to 9.2, while moisture content changed (8.5–79.5%). Crude protein content remained relatively stable during treatment (52.7–58.7% db up to 30 min), whereas crude fat decreased significantly by 27.2% after 40 min of cooking. Chitin content increased by up to 47.4% at short treatment times (0–10 min), while calcium concentration increased progressively with cooking time, reaching 514 mg/100 g at 40 min. FTIR analysis revealed an increase in β-sheet structures from 57.1% (control) to 68.8% (40 min) and a simultaneous reduction in random coil content from 14.3% to 6.3%, which correlated with reductions in free amino nitrogen (48.8 to 8.9 mg/g) and *in vitro* protein digestibility (88.7% to 74.4%). Tortillas supplemented with alkali-treated *S. purpurascens* (T0) showed higher protein content (10.4 vs 7.2% db), increased lysine contribution (56.3 vs 45.6% of FAO requirement), and a higher PDCAAS (67.1 vs 51.1%) compared to control tortillas. Textural analysis indicated softer but more fragile tortillas, with lower breaking force after 24 h of storage (0.252 vs 0.414 kg), suggesting delayed structural stiffening. These results demonstrate that alkaline treatment modifies the functional and nutritional properties of *Sphenarium purpurascens*, enabling its successful incorporation into maize tortillas. Applying nixtamalization to an insect-based protein matrix represents a novel processing strategy to improve protein quality and diversify edible insect applications in staple foods.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Processing the black soldier fly larvae as ingredient in extruded corn snacks

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Edible insects are increasingly being studied and promoted as a valuable nutritional resource. *Hermetia illucens* (black soldier fly, BSF) is a promising candidate for commercial insect-derived protein production owing to its high protein content and a favourable essential amino-acid profile. While BSF biomass is currently deployed mainly in animal feed, growing nutritional and processing evidence indicates substantial potential for its sustainable incorporation into food systems. The aim of this study was to obtain protein-enriched flour from BSF larvae by supercritical carbon dioxide extraction and to evaluate its inclusion in extruded products. The obtained BSF flour contained 60% protein, 2.7% fat, 12.6% ash, 8.9% fibre and 9.2% carbohydrates. Glutamic and aspartic acids were the most abundant amino acids (6.9 and 6.2%, respectively) followed by leucine (4.5%) and lysine (4.2%). BSF flour was incorporated into corn grits at 5, 10 and 20% substitution level, and extrudates were produced using a twin-screw extruder. Snacks without BSF flour served as the control. Addition of BSF flour increased protein content up to 2.2-fold and mineral content up to 3.5-fold compared with the control. With BSF flour addition, the bulk density and hardness of extrudates increased. Colour changes were observed: higher BSF flour levels produced darker products, and the colour parameters a^* and b^* shifted toward red and blue, respectively. Sensory evaluation indicated that formulations containing 5 and 10% BSF flour showed slight reductions in overall score (29 and 27 points, respectively) compared with the control (30 points), while the sample with 20% addition recorded the lowest overall score (24 points). The results of this study may contribute to optimising the acceptability of BSF in food products. The project OptiFood is supported by funds of the Federal Ministry of Agriculture, Food and Regional Identity (BMLEH) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Safety assessment and characterisation of silkworm pupae as a potential novel food in Europe

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Silkworm pupae are a common by-product of sericulture and provide a source of proteins, polyunsaturated lipids and bioactive compounds. In Asia, silkworm pupae have a long tradition of consumption as food; however, in Europe — despite a long tradition of silkworm rearing — they have not been widely used. For this reason, any potential authorisation of silkworm pupae as a novel food in Europe must follow the procedure established by the EU Novel Food Regulation. In accordance with the technical and safety requirements set by European legislation, a dedicated processing method and a full characterisation of a potential novel food product were carried out. The suitable pupae came from second-grade cocoons not intended for reeling. The pupae were removed alive from the cocoons and euthanised in accordance with principles of animal welfare applicable to insects. The final product consisted of dehydrated silkworm pupae that were fully characterised in terms of composition and potential hazards. All chemical and biological safety parameters remained well below the limits established for other insect-based products authorised in the European Union. Regarding composition, proteins were the main nutrient, accounting for 45.9–50.4 g/100 g, while fats represented the second most abundant macronutrient, with values ranging from 32.6 to 42.7 g/100 g. With respect to contaminants, cadmium and mercury levels were below the limits of quantification, whereas lead concentrations ranged from 0.013 to 0.04 mg/kg and arsenic from 0.039 to 0.275 mg/kg. Dioxins and dioxin-like PCBs ranged from 0.185 to 0.679 pg WHO-PCDD/F-PCB-TEQ/g. All microbiological indicators investigated in the novel food were below the limits of quantification. The only exception was *Bacillus cereus*, which reached 230 CFU/g in a single batch. Shelf-life analyses covered eight months and showed no significant changes in microbial load. Regarding oxidative stability, peroxide values ranged from 0.6 to 2.9 mEq O_2/kg at time zero, increasing to 1.7–7.3 mEq O_2/kg at the eighth month. Apart from possible allergenicity, the main potential hazards regard the presence of heavy metals, whose levels strongly depend on soil contamination affecting mulberry leaves, and which therefore require future monitoring. Overall, our results demonstrate that the developed product represents a nutritious and safe alternative food source, with the added benefit of deriving from an environmentally sustainable agricultural practice. Moreover, its use could increase the value of sericulture by further enhancing traditional agricultural activities in Europe.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Fate of chitin from different insect fractions during *in vitro* human gut fermentation

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This study explores the behaviour of insect-derived ingredients in the human gastrointestinal tract, focusing on chitin, a structural polysaccharide of the exoskeleton of arthropods. As insects are increasingly recognised as a sustainable food source, understanding chitin digestion and its impact on gut microbiota is essential for developing health-promoting food products. *In vitro* digestion and 48-h fermentation experiments were conducted on several insect species (*Acheta domesticus*, *Tenebrio molitor*, and *Hermetia illucens*) and their chitin-rich fractions (defatted insects, cuticle, and chitin). Additionally, a 15-day experiment employing the Simulator of Human Intestinal Microbial Ecosystem (SHIME®) was performed with a basal diet supplemented with 30% previously digested insect ingredients. Chitin was resistant to digestion and not rapidly fermented under short-term fermentation, as reflected by negligible short and branched fatty acids (SCFAs, BCFAs), and ammonia production. However, relevant metabolic and microbial changes were observed in the insect-based diet during the long-term SHIME® experiment. These changes became evident after 5 days of treatment, indicating gradual microbial adaptation. Notably, the insect-supplemented diet led to shifts in SCFA profiles, with reduced acetate (24 mM and 38 mM in the proximal (PC) and distal colon (DC) compared to 42 mM and 53 mM in the control diet) and increased butyrate (9 mM both in PC and DC compared to 1 mM and 4 mM). In addition, higher ammonia levels and BCFAs (3 mM both in PC and DC compared to<1 mM in the control diet), reflected enhanced proteolytic fermentation, likely related to the progressive degradation of chitin-protein complexes. Furthermore, the microbial community composition evolved over time, with an increase in beneficial bacteria, particularly members of the family *Bifidobacteriaceae*, and the emergence of new bacterial populations (e.g. *Synergistaceae* family), suggesting adaptation and possible selective stimulation of gut bacteria. Insect chitin represents a novel dietary fibre with possible prebiotic properties that can modulate gut microbiota over extended periods. These findings contribute to a better understanding of insects as a sustainable and health-promoting diet, encouraging further research into the functional applications of insect-based foods.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Functional peptide fractions from black soldier fly protein: bioactivity, selectivity and gut microbial metabolism

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Black soldier fly larvae (BSFL) protein is an emerging sustainable ingredient with relevance in food and feed systems. Beyond its nutritional value, enzymatic processing and gastrointestinal digestion can release bioactive peptides with functional and gut-relevant properties; however, how different processing routes influence biological and microbial responses remains unclear. This study evaluated how enzymatic hydrolysis and simulated digestion affect the functional activity, cellular selectivity, and gut fermentation behaviour of BSFL protein–derived peptide fractions. BSFL protein was hydrolysed using Alcalase, bromelain, trypsin, and a standardized *in vitro* gastrointestinal digestion model (INFOGEST), followed by molecular weight fractionation. Hydrolysates were assessed for antioxidant capacity, angiotensin-converting enzyme (ACE) inhibitory activity, and selective cellular responses using human colon cancer and healthy intestinal cell models. Selected fractions were further evaluated using an *in vitro* human gut fermentation system to quantify short-chain fatty acid (SCFA) production. Simulated gastrointestinal digestion produced the highest extraction yield (approx. 65%) and degree of hydrolysis (approx. 43%), compared with enzymatic treatments (30–40% yield; 20–27% hydrolysis). Hydrolysis significantly enhanced antioxidant activity, with low-molecular-weight peptide fractions exhibiting 1.5–1.8-fold higher activity than native protein. Several fractions demonstrated notable ACE-inhibitory activity, reaching 50–60% inhibition. Bromelain-derived peptide fractions reduced colon cancer cell viability to approximately 45–55% at 1 mg/ml while maintaining healthy cell viability above 95%, accompanied by increased activation of intrinsic apoptosis markers. *In vitro* gut fermentation of selected peptide fractions resulted in substantial SCFA production, with acetate, propionate and butyrate concentrations ranging from approx. 165–695 μM, 568–791 μM and 401–691 μM, respectively. INFOGEST and enzyme-derived fractions promoted higher total SCFA production than raffinose and control substrates while maintaining moderate levels of branched-chain fatty acids, indicating active yet controlled microbial protein fermentation. Overall, these findings demonstrate that BSFL-derived peptide fractions are functionally distinct and strongly dependent on processing strategy. By linking enzymatic processing, peptide bioactivity, and gut microbial metabolite production, this work highlights the potential of insect-derived proteins as multifunctional ingredients for sustainable food and feed applications.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Extrusion of insect-plant protein blends: from insect ingredients to structural diversity

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To support development of insect food products with high added value, innovative processing using insect fractions as ingredients needs to be explored. Combining insect ingredients with other alternative protein sources in extrusion processes is one way to diversify of insect food applications. This work integrates studies on incorporation of insect-derived ingredients into extruded protein matrices to demonstrate how ingredient composition and moisture conditions determine extrudate structure and quality. High-moisture extrusion studies focused on fibrous structure formation through the partial substitution of soy or pea protein with different yellow mealworm fractions, including whole meal and soluble and insoluble protein fractions, at inclusion levels up to 50%. Increasing insect inclusion level altered extrudate composition and led to reduced texturization, fibre strength, and structural anisotropy while increasing extrudate darkening. Distinct fraction-dependent effects were observed, with chitin-rich fractions constraining the development of continuous fibrous networks. Across experiments, ingredient composition showed a stronger influence on structural outcomes than processing conditions and screw configuration. Insect-plant protein high moisture extrudates can serve as structured protein matrices for applications such as meat analogues and other fibrous protein foods. Low-moisture extrusion tests investigated the partial replacement of lentil flours with black soldier fly larvae ingredients at inclusion levels between 10 and 25%. Across formulations, changes in hardness, water absorption capacity, expansion ratio, and colour were primarily associated with the inclusion of insect ingredients. These extrudates are suitable for applications where compact, shelf-stable products with controlled hydration properties are required, such as dry protein ingredients, snacks, or feed formulations. The results demonstrate how systematic variation in insect ingredient composition and extrusion moisture conditions enables a wide range of extrusion outcomes, from compact low-moisture products to anisotropic fibrous structures.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Production and characterisation of extruded snacks with incorporation of mixtures of house crickets and lupin beans

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Food products containing alternative proteins are an expanding market in the agri-food sector, but their functionality and sensory properties still affect their success. This study aimed to develop and characterise extruded snacks incorporating controlled blends of whole house cricket (*Acheta domesticus*) flour (WC) and lupin bean protein concentrate (L), and to subsequently fit an appropriate statistical model to the resulting data to support product optimisation. A 13-point experimental mixture design was used, with corn flour as the main component (minimum 85%) and L and WC added to a maximum of 15%. Extruded samples were characterised for their physical and compositional properties (e.g., sectional expansion index (SEI), porosity), colour, and texture parameters. These data were processed and adjusted to optimal response surface models. All samples expanded acceptably; however, protein incorporation significantly decreased SEI, particularly in samples with 15% WC or 10% WC + 5% L (7.28 and 6.86, respectively; control 10.22). Most water-related properties were similar to control, especially the swelling index (ca. 6-7 ml/g; control 6 ml/g), and the water content (between 0.030–0.040 g/g; control 0.041 g/g), while porosity increased, particularly in samples with higher protein content (e.g., 2.5% WC + 10% L: 0.94; 5% WC + 10% L: 0.94; control: 0.91). Regarding colour parameters, samples with increased protein content became darker (lower L^* and b^* values), a known effect of adding dry crickets, with no impact of lupin incorporation. Texture parameters were largely unaltered by the different blends, resulting in products like the control, suggesting an overall positive outcome. Comparing different statistical models and their quality of adjustment, a cubic model was chosen as an overall optimal response surface, which can be useful in further product development. All in all, the study demonstrates that snacks enriched with cricket and lupin proteins can be produced with desirable structural, optical, and textural attributes, within feasible and permitted protein inclusion levels.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Mono- and disaccharide composition of insects and their suitability for individuals with disaccharide intolerance

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Insects are gaining attention as a sustainable and nutritionally valuable food source, yet information on their carbohydrate fraction, especially simple sugars, is still limited. The present study focused on the determination of selected mono- and disaccharides in a diverse set of insect species and on evaluating their suitability for consumption by individuals with disaccharidase deficiencies. A total of nineteen insect samples covering five taxonomic orders, various developmental stages and both sexes were analysed. Concentrations of trehalose, glucose, fructose, lactose, sucrose and maltose were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), while total soluble saccharides were assessed by the anthrone method. The combined content of quantified mono- and disaccharides ranged from 49 to 1675 mg/100 g of lyophilised insect material, whereas total soluble saccharides reached 253–5403 mg/100 g. Trehalose represented the dominant disaccharide in most species, with maximum levels of 495 mg/100 g in *Hermetia illucens* larvae and 215 mg/100 g in *Tenebrio molitor* larvae. Glucose concentrations varied markedly among species, peaking at 1328 mg/100 g in subadult *Locusta migratoria*. Lactose was absent or detected only at trace levels (<5 mg/100 g) in most samples; an exception was observed in *Musca domestica* larvae, where 84 mg/100 g was found, likely reflecting residual dietary sources. Statistical analysis confirmed a significant influence of species, developmental stage and sex on saccharide composition ($p < 0.001$). In general, the low levels of lactose and sucrose observed in most insect species indicate favourable suitability for individuals affected by common disaccharide intolerances. In contrast, the highly variable trehalose content suggests that certain species may pose a risk for individuals with trehalase deficiency. These findings contribute to a better understanding of carbohydrate composition in insects and support informed dietary recommendations regarding insect consumption for people with disaccharidase-related intolerances. Acknowledgments: The study was supported by the METROFOOD-CZ research infrastructure project (MEYS grant number: LM2023064), including access to its facilities.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Chitin-rich black soldier fly larvae fractions as functional ingredients for hyperglycaemia management

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Black soldier fly larva (*Hermetia illucens*, BSFL) represents a sustainable and underutilised bioresource with growing relevance for functional foods and nutraceutical applications. Chitin extracted from BSFL has previously demonstrated antioxidant and anti-inflammatory properties, but its potential in managing hyperglycaemia and type 2 diabetes mellitus through carbohydrate-digesting enzyme inhibition and antiglycation activities remains underexplored. This study compared the inhibitory effects of chitin-rich BSFL fractions, obtained through chemical and microwave-assisted extraction methods, on α-amylase, α-glucosidase, and protein-bound fluorescent advanced glycation end-products (AGEs) inhibition. Defatted BSFL samples were prepared as three fractions: untreated protein/chitin mix, chemically extracted fractions and microwave-assisted extracts. α-amylase and α-glucosidase inhibitory activities were assessed *in vitro* using the 2-chloro-4-nitrophenyl α-D-maltotrioside reagent and the p-nitrophenyl-α-D-glucopyranoside substrates, respectively. Antiglycation activity was evaluated by measuring the formation of protein-bound fluorescent AGEs levels in an *in vitro* model system. The untreated protein/chitin mix showed significantly stronger α-amylase inhibition (IC_{50} = 3780.00 ± 58.59 μg/ml) compared with microwave-assisted extracts (IC_{50} = 6423.00 ± 380.10 μg/ml, $p < 0.05$). Chemically extracted fractions (IC50 = 5190.00 ± 1690.00 μg/ml) showed no significant difference when compared with protein/chitin mix and microwave-assisted extracts, respectively. In contrast, α-glucosidase inhibition showed limited activities. The chemically extracted fraction demonstrated the strongest antiglycation activity (IC50 = 239.40 ± 19.45 μg/ml), which was significantly ($p < 0.05$) lower than both protein/chitin mix (945.70 ± 49.70 μg/ml) and microwave-assisted extracts (1065.70 ± 31.64 μg/ml), and notably lower than the positive control aminoguanidine (IC_{50} = 558.40 ± 23.70 μg/ml). Overall, chitin-rich fractions derived from BSFL exhibited notable α-amylase inhibitory and antiglycation activities, supporting their potential role in hyperglycaemia management. While extraction process reduced the α-amylase inhibition when compared to untreated samples, chemical extraction significantly enhanced the antiglycation activity. These findings highlight BSFL as a sustainable ingredient for future functional food and health-oriented applications.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Papain-mediated hydrolysis of yellow mealworm (*Tenebrio molitor*) proteins for hypoallergenic ingredient development

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Edible insects are emerging as sustainable alternative protein sources due to their high nutritional value, reduced environmental footprint and versatility in diverse food applications. However, their incorporation as ingredients in conventional food matrices where their presence may go unnoticed, raises safety concerns, primarily related to allergenic potential. In particular, yellow mealworm (*Tenebrio molitor*) contains pan-allergenic proteins (e.g., tropomyosin) which are homologous to major crustacean allergens and may induce IgE-mediated reactions in sensitized individuals due to cross-reactivity. This work proposes a novel papain-mediated hydrolysis strategy to mitigate the cross-reactivity of yellow mealworm flour. Papain, a cysteine endopeptidase extensively used in the food industry, was applied in a simple and cost-effective process involving a 3-hour incubation at 65 °C of insect flour suspended in water, using enzyme concentrations ranging from 1.25 to 2.50 U/ml. Under these conditions, extensive proteolysis was achieved, resulting in the disruption of conformational and linear IgE-binding epitopes. Immunoblotting analysis demonstrated complete elimination of IgE reactivity when tested with sera from crustacean-allergic patients. In silico hydrolysis simulation further corroborated the degradation of known allergenic epitopes within allergenic proteins. Collectively, these results demonstrate that the developed method offers a technologically feasible strategy for producing hypoallergenic, protein-rich, insect-derived ingredients suitable for incorporation into a wide range of food products. The proposed methodology has been protected as an innovation through a provisional patent application (PT120590) filed with the National Institute of Industrial Property (INPI) (Texeira *et al.*, 2025). Reference: Teixeira, C.S.S., Villa, C., Carriço-Sá, B., Biltes, R., Dias, C., Costa, J. and Mafra, I., 2025.750 Method for producing an edible insect-based product. Provisional patent application PT120590 filled in 15/09/25. Portuguese Institute of Industrial Property. Patent pending. Funding: This work received financial support from European Union, FEDER, through the project EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX), ALLEVIATE (2023.12193.PEX), UID/50006/2025, and IMMUNOGATE (COMPETE2030-FEDER-00848700).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Biocomputing analysis of allergenic glutathione transferases in edible insects

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Insect proteins are increasingly explored for food, feed and biotechnological applications, raising the need for systematic allergenicity assessment. This study employed advanced bioinformatic tools to investigate the allergenic potential of Glutathione Transferases (GSTs) from selected insect species. Protein sequences from *Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus* and *Alphitobius diaperinus* were analysed against curated databases of known allergens to identify sequence similarity patterns indicative of IgE-binding capacity. GST sequences were evaluated using established similarity thresholds, with values ≥90% considered indicative of a high likelihood of allergenicity. The analysis revealed that *T. molitor* GSTs showed the most pronounced allergenic signatures, with two sequences exceeding the 93% threshold relative to GST allergens from *Blattella germanica*. This high level of homology strongly suggests structural conservation of IgE-reactive epitopes and underscores the potential of *T. molitor* GSTs to elicit allergic responses. Although *L. migratoria* did not present sequences surpassing the cutoff, several GSTs exhibited high similarity values, indicating partial conservation of allergenic domains and possible immunological relevance. Phylogenetic reconstruction supported these findings by demonstrating evolutionary relatedness among insect GSTs, further implying epitope preservation across species. In contrast, GSTs from *A. domesticus* and *A. diaperinus* showed weaker homology, suggesting comparatively lower allergenic potential based on current computational evidence. Collectively, these findings indicate that insect-derived GSTs, particularly two isoenzymes from *Tenebrio molitor* and *Locusta migratoria*, display strong molecular signatures consistent with potential allergenic activity. Based on these results, the corresponding GST-encoding genes from *T. molitor* and *L. migratoria* were successfully cloned into T7 promoter–driven expression plasmids optimised for heterologous expression in *Escherichia coli*. Both enzymes were subsequently expressed as soluble recombinant proteins, enabling their isolation in quantities suitable for downstream analyses. Ongoing and future work will focus on the comprehensive biochemical and immunological characterisation of the recombinant GSTs, with particular emphasis on IgE epitope mapping and the evaluation of immunoreactivity using sera from sensitized human donors. These investigations are expected to experimentally validate the predicted allergenic potential inferred from sequence-based analyses and to provide critical data for allergenicity risk assessment.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Evaluation of the allergenic potential of novel insect proteins by IgE cross-reactivity

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The increasing adoption of sustainable alternative protein sources, such as edible insects in both human and animal diets raises important questions regarding their risk-assessment. One of the most relevant issues is the potential to elicit allergic reactions in individuals sensitized to phylogenetically related protein sources. This study aimed to assess the cross-reactive potential of insect-derived proteins using a combined immunological and proteomic approach. Sera from 48 patients with documented allergies to shrimp and/or house dust mites (HDM) were used to evaluate IgE cross-reactivity against protein extracts from two insect species, i.e., the yellow mealworm, *Tenebrio molitor* L., and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Proteins present in immunoreactive bands were identified by high-resolution mass spectrometry (HPLC-HRMS) and their amino acid sequences were retrieved and compared against the AllergenOnline database using the dedicated search tool (www.allergenonline.org). Sequence alignments were evaluated according to internationally accepted criteria for allergenicity assessment, with significant matches defined as a minimum of 35% sequence identity over at least 80 contiguous amino acids (FAO/WHO recommendations). Our results showed that insect proteins exhibited pronounced IgE cross-reactivity with common invertebrate allergens, such as tropomyosin, paramyosin, troponine, myosin and arginine kinase. These findings suggest that exposure to these novel food sources could potentially trigger allergic reactions in patients with established sensitisation patterns, highlighting the need of integrating allergenic risk assessment into the safety evaluation of novel protein sources. Overall, this work supports the development of evidence-based strategies to ensure a safe introduction of sustainable alternative proteins into the animal and human diets.
Acknowledgement: Financial support for this research has been provided by PRIMA under grant agreement No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Food allergen carryover in the edible insect supply chain: investigation in a pilot plant

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The use of insects as source of food has an important environmental and economic benefit: for these reasons all insect-based foods are included among the categories of food constituting novel foods. Insects may be an alternative food source but consider insects as food requires scrutiny due to risk of allergens. The aim of this work was to evaluate if food allergens could be vehiculated by edible insects from the substrate on which they are grown to the final product. *Hermetia illucens* larvae were grown on substrates containing 10% of each of the following allergens: peanuts, celery, soy, almonds and gluten. At the end of the feeding period, larvae were sampled at T0 (end of feeding), T1 (24 h fasting), T2 (48 h fasting) and subsequently frozen, dried and ground. Additionally, for each of the three time points, a negative control group was tested, consisting of larvae reared on substrates free of food allergens. Larva samples reared on substrates with and without allergens were analysed to evaluate the presence of allergen residues using: i) real-time PCRs (SPECIALfinder MC, Generon) and ii) enzyme-linked immunosorbent assays (ELISA RIDASCREEN® Gliadin, r-Biopharm). Real-time PCR for the detection of the soy allergen showed positive results in all analysed samples, (mean Ct: 28.84 at T0, 29.4 at T1, 30.95 at T2). Similarly, the celery target was detected in 7/9 replicates tested (mean Ct: 26.74 at T0, 26.90 at T1, 29.77 at T2), while the almond target was identified in one replicate at T0 (Ct 33.96) and in two replicates at T1 (mean Ct 34.01). Negative results were obtained for larva samples treated with the peanut allergen. As expected, the negative controls showed no fluorescence signals for the targets investigated. Values<5 mg/kg were obtained at T0, T1, and T2 for the gluten allergen as well, using the ELISA test. Insects may represent an alternative food source; however, their use requires careful evaluation due to the potential presence of allergens. The results obtained in this study highlight that if insects are fed on substrates containing allergens, these allergens can be transferred to the insects and, consequently, to the food products derived from them. To reduce the 'allergy risk' for final consumers, it is essential to consider using allergen-free growth substrates or to include appropriate precautionary labelling that refers to allergens potentially present even in trace amounts. Acknowledgement: Project funded by the Italian Ministry of Health: IZSPRC, CUP J19I23001630001

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Edible insect-derived ingredients for type 2 diabetes: the DiBaN project

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Type 2 diabetes (T2D) is a chronic metabolic disorder characterised by insulin resistance and hyperglycaemia, with intestinal dysbiosis emerging as a key contributing factor, often linked to poor dietary habits. In this context, edible insects are gaining attention as sustainable and innovative sources of nutrients and health-promoting bioactive compounds. The DiBaN project aims to develop novel ingredients from bioactive-enriched edible insects to counteract dysbiosis and improve metabolic health related to T2D. *Acheta domesticus* has been selected as the model species for this project due to its high nutritional value and its approval for human foods. Specifically, DiBaN investigates how the nutritional and metabolic status of insects influences the subsequent bioactivity of insect-derived products, and how this can be boosted through dietary supplementation with specific bioactive and sustainable diets, such as microalgae. As general approaches of DiBaN, insects reared on conventional or microalgae-enriched diets are being processed into conventional products (full-fat powder, defatted powder or oil). Furthermore, advanced extraction techniques are being applied to develop innovative insect-based bioactive extracts. The nutritional composition and bioactivity of the products are being evaluated for vitro antioxidant, anti-inflammatory, anti-diabetic and hypolipidemic properties. The most promising insect-derived products are being assessed in *ex vivo* models (organoids and organ-on-a-chip). In parallel, two *in vivo* intervention studies (mice and human) are investigating the personalized metabolic and microbiome responses to dietary supplementation with *A. domesticus* powder. Finally, all generated data will be integrated into the development of a prototype of an AI-based predictive tool capable of forecasting personalized responses to nutritional interventions based on insect-derived ingredients. Therefore, the DiBaN project pioneers a new strategy to tackle T2D by exploiting the potential of edible insects as tailored sources of bioactive compounds capable of modulating the gut microbiome–host metabolic axis through targeted feeding strategies of the insects. Acknowledgement: This project is funded by the European Union under grant agreement No 101162517, call HORIZON-EIC-2023-PATHFINDERCHALLENGES-01.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Identification of bioactive peptides with potential human health applications from black soldier fly larvae

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Black soldier fly larvae (BSFL) are increasingly being recognised as an alternative source of food protein among other edible insects. However, little research has been conducted into the potential beneficial effects of consumption of BSFL proteins. This study investigated the potential of BSFL as a source of bioactive peptides with antidiabetic, antihypertensive, and antioxidant properties combining *in silico* and experimental processes. The *in vitro* approach involved collecting, washing and starving the BSFL for 24 h before producing protein extracts. Two sample types were generated: one dried by microwave and the control which was not treated. Both samples were stored frozen at –80 °C before analyses with *in vitro* bioassays and advanced mass spectrometric analysis to identify proteins via UniProt databases. The *in silico* approach involved using several bioinformatic tools to simulate *in vivo* digestion of BSFL proteins by pepsin and trypsin, as well as determining the bioactivity of the resulting peptides. Finally, molecular docking experiments were performed to establish the extend of possible bioactivity. A total of 73 bioactive peptides were identified during the *in silico* process, focusing only on the ten most abundant proteins identified in BSFL by mass spectrometry. Most of these peptides are derived from hexamerin/hemocyanin and possess antihypertensive properties (n = 64). Other identified properties include antidiabetic, DPP-IV inhibition and antioxidant activity. Antioxidant potential was evaluated using the DPPH assay, with the non-treated BSFL protein extract exhibiting 71.2% radical scavenging capacity at a concentration of 30 μg/ml, decreasing to 14.3% at 3.75 μg/ml. Computational molecular docking experiments using the HADDOCK 2.4 cloud server produced HADDOCK scores ranging from –16.4 to –70.2, indicating high to very high binding affinities to suitable targets. These finding are supported through calculation of intermolecular interactions and the prediction of protein-peptide binding energies, resulting in ΔG values ranging from –9.9 to –6.4 kcal/mol, crucial for high affinity binding to target proteins. This study demonstrates that the BSFL are not only rich in proteins, but digestion potentially generates a promising panel of bioactive peptides, with antidiabetic, antihypertensive and antioxidant properties. More detailed *in-vitro* assay will support these finding.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Bioactive peptides from *Acheta domesticus*: in silico, *in vitro*, and cellular cardiometabolic assessment

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Entomophagy is emerging as a sustainable and nutritious alternative protein source. Beyond their nutritional value, insect-derived peptides released during gastrointestinal (GI) digestion may confer health benefits, with antihypertensive activity, mediated through inhibition of somatic angiotensin-converting enzyme (sACE), being among the most widely reported. This study began with an in silico approach to simulate the GI digestion of house cricket (*Acheta domesticus*) proteins and identify peptides with potential sACE-inhibitory activity. Molecular docking was then performed to evaluate binding interactions between the resulting peptides and sACE catalytic domains. Peptides AVQPCF, CAIAW, IIIGW, QIVW, PIVCF and DVW exhibited high docking scores, suggesting the potential to inhibit sACE activity. Those peptides were subsequently validated *in vitro*. LC-MS/MS confirmed their presence in *A. domesticus* digesta, and enzymatic assays demonstrated potent sACE-inhibitory activity for AVQPCF, PIVCF and CAIAW, with IC_{50} values of 3.69 ± 0.25, 4.63 ± 0.16 and 6.55 ± 0.52 μM, respectively. The digesta also exhibited significant sACE inhibition (IC_{50} = 77.1 ± 11.8 μg protein/ml extract). Finally, the peptides were assessed in Caco-2 cells to evaluate intestinal absorption, cytotoxicity, barrier integrity, and modulation of gene expression markers associated with diabetes (DPP-4, SGLT1) and hypertension (sACE, ACE2). All peptides were non-cytotoxic up to 2 mM; however, DVW and PIVCF disrupted epithelial integrity. Only DVW was transported across the epithelium intact. Peptides DVW and PIVCF significantly downregulated SGLT1 expression (0.42- and 0.52-fold, respectively), suggesting a potential antidiabetic mechanism mediated by reduced intestinal glucose absorption. Taken together, this work demonstrates the bioactivity of *A. domesticus*-derived peptides, highlighting the potential of this insect as a multifunctional ingredient for promoting cardiometabolic health. Funding: This work received financial support from European Union, FEDER, through the project EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX), ALLEVIATE (2023.12193.PEX), UID/50006/2025, and IMMUNOGATE (COMPETE2030-FEDER-00848700).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Development and quality evaluation of a novel protein bar enriched with food-grade roasted *Tenebrio molitor* powder

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Insect-based food consumption is a rising global trend, driven by sustainability, circular economy, nutrition and food security. This study aimed to develop protein bars incorporating roasted *Tenebrio molitor* (TM) larvae flour and evaluate their physicochemical, nutritional and sensory properties. Initially, the effect of three roasting temperatures (90,110, 130 °C) on colour, water-holding capacity (WHC), oil-holding capacity (OHC), swelling capacity (SWC), Thiobarbituric acid reactive substances (TBARS), and total phenolic content (TPC) of the flour was examined. Protein bars were then developed with 12% (12MB) and 15% (15MB) roasted TM flour (roasted at 130 °C), blended with oats, chickpea, peanuts, coconut treacle, salt, vanilla and spices without added sugar. The bars were analysed for proximate composition, TBARS, TPC, and consumer sensory acceptance compared to control bar without TM (CB). Colour, WHC, OHC, SWC and TBARS of roasted flours were not significantly different ($p > 0.05$), but TPC was statistically different ($p < 0.05$) among treatments remaining within the acceptable range (5–20 mg GAE/g). Considering the pleasant aroma, 130 °C was selected for the preparation of protein bars. The crude protein content was 12.9% in the CB, while in 12MB and 15MB it was 18.9 and 21.5%, respectively, significantly higher ($p < 0.05$) compared to CB. Crude fat contents were 10.5, 12.5 and 12.6% for CB, 12MB and 15MB, respectively. Crude fibre contents were 2.9% (CB), 5.5% (12MB) and 4.0% (15MB). Both crude fat and fibre contents showed significantly higher values ($p < 0.05$) in TM contained bars. TBARS of 12MB showed a significantly low ($p < 0.05$) value, but both 12MB and 15MB were in the acceptable level of ≤2.0 mg MDA/kg. TPC values did not differ significantly among three formulations ($p > 0.05$). In the sensory evaluation, a considerable consumer segment (20/31) was interested in TM included protein bars in terms of all sensory attributes, in particular 15MB was the most preferred ($p < 0.05$), showing strong consumer acceptance. These findings demonstrate that appropriate thermal processing, such as roasting, allows TM flour to be successfully included in protein bars, supporting the development of sustainable, high-protein foods with favourable consumer acceptance.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Integration of *Tenebrio molitor* flour in protein bars: from formulation to industrial scale-up and market comparison

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Consumer demand is shifting toward protein-rich snacks that combine health appeal with improved sustainability accelerating interest in novel protein ingredients such as insects. These ingredients provide nutrient-dense alternatives with low environmental impact. This study aimed to develop protein bars incorporating *Tenebrio molitor* larvae flour (TM) at different concentrations levels and to evaluate and compare performance across successive stages of product development. Four variants of protein bars were formulated, containing 0, 5, 10 or 15% (w/w) TM. Sensory screening enabled the down-selection of the 10 and 15% (w/w) formulations, which progressed to further technological optimisation. This was supported by sensory mapping that clarified the drivers of liking, indicating that consumer acceptance was associated with attributes such as a brownish appearance, intense odour, crunchy texture and earthy-bitter flavour. The formulation containing 10% (w/w) TM aligned with these attributes and was positioned close to the acceptance vector. Although the 15% (w/w) formulation also presented a positive acceptability score, it was characterised by less favourable textural attributes, particularly a pronounced grainy and powdery texture. Therefore, these formulations were subsequently subjected to texture-focused reformulation. An alternative processing technique (NP) was evaluated, applied exclusively to the insect flour (freezing and grinding), in comparison with the flour as originally supplied. Texture analysis showed that the NP consistently improved firmness and toughness compared with the old method, although at the cost of markedly increasing adhesiveness, effect that aligned with sensory results, which identified the 10% (w/w) NP formulation as the preferred sample and the 15% (w/w) NP formulation as the least appreciated. Based on that the 10% (w/w) TM formulation was selected for industrial Scale-up. Following scale-up, this bar was benchmarked against tree commercial bars, including one containing insect-derived ingredients and two conventional bars without insect components. This final sensory test validates that the formulation obtained achieves sensory performance comparable to commercial products. Overall, this work highlights the importance of an integrated approach combining formulation design, sensory evaluation, process optimisation, and market benchmarking in the development insect-based functional foods. Acknowledgement: This work was funded by InsectERA (No. C644917393-00000032), WP InFood, supported by Next Generation EU and Portugal's PRR under "Agendas para a Inovação Empresarial" (C5).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Acheta domesticus or *Tenebrio molitor*? Species-dependent effects of insect powders on wheat bread quality

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Despite the growing interest in insect-enriched bakery products, direct comparative studies assessing how the addition of different insect species affects bread quality remain limited. This study aimed at comparing the effects of adding *Acheta domesticus* or *Tenebrio molitor* powders as partial replacements for wheat flour on bread colour, baking loss, crumb hardness, and sensory acceptance. Wheat flour was partially replaced with *Acheta domesticus* or *Tenebrio molitor* powder at levels of 5, 10, 15 and 20%. The breads were prepared using ingredients, including oil, salt, sugar, and dried yeast. Colour parameters (L^*, a^*, b^*), crumb hardness (penetration test), baking loss, five sensory attributes (flavour, aroma, hardness, chewiness and gumminess), and overall acceptability were evaluated using a 9-point hedonic scale. Statistical analysis was performed using two-way ANOVA. Both insect species and inclusion level significantly affected most quality parameters. Increasing insect content decreased lightness (L^*), with a more pronounced darkening effect observed in *Acheta domesticus*. Colour parameters a^* and b^* differed significantly between species, indicating a distinct influence of insect raw material on bread colour. Crumb hardness depended on both species and inclusion level, though it was less pronounced than colour and sensory changes. Baking loss varied among formulations, without a clear species-dependent trend. Sensory evaluation revealed significant differences between insect species. Breads containing *Acheta domesticus* received higher scores for flavour and overall acceptability than those with *Tenebrio molitor*. Increasing insect addition led to a gradual decrease in sensory acceptance regardless of species. *Acheta domesticus* showed a more favourable impact on sensory attributes, which may be related to differences in chemical composition and interactions with the gluten matrix. In contrast, *Tenebrio molitor* had a greater effect on colour and consumer acceptance at higher inclusion levels. Conclusions *Acheta domesticus* demonstrates greater application potential than *Tenebrio molitor*, particularly at moderate inclusion levels (5–10%), highlighting the importance of species-oriented formulation strategies in the development of insect-based bakery products.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Black soldier fly oil as a cocoa butter alternative in chocolate

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Cocoa butter is a key ingredient in chocolate, but its production faces numerous challenges, creating a need for sustainable alternatives. Previous studies have shown that coconut oil can be used as a replacer for cocoa butter in chocolate. The lipid fraction of *Hermetia illucens* larvae (black soldier fly, BSFL) shares many similarities with coconut oil in terms of fatty acid composition and may therefore also be suitable as a cocoa butter replacer in chocolate. This study aimed to investigate the feasibility of partial replacement (5 and 10% of the total mass of chocolate) and full replacement (15% of the total mass of chocolate) of cocoa butter with BSFL oil in chocolate, and to evaluate the effects on texture, melting behaviour, colour, and volatile profile. All results were compared with chocolate produced using the same replacement experimental design but formulated with coconut oil instead of BSFL oil. Differential Scanning Calorimetry showed that the incorporation of BSFL oil reduced the melting point of chocolate from 32.1 °C (no replacement) to 26.7 °C and 26.3 °C at full replacement of cocoa butter with BSFL and coconut oils, respectively. The breaking force of chocolate decreased with increasing levels of BSFL oil, from 6470 g (no replacement) to 1857 g at full replacement. The reduction in breaking force was even more pronounced in chocolate with full replacement of cocoa butter by coconut oil (1262 g). Chocolate colour was assessed using an IRIS visual analyser that showed differences between control chocolate and all formulations with cocoa butter substitution either with coconut oil or BSFL oil; however, these differences were not visually detectible. Analysis of the volatile profile revealed largely similar results across all samples. However, full replacement with black soldier fly lipids showed additional presence of 2-heptanol and linalool at low concentrations. In conclusion, BSFL oil showed promising potential as a partial replacer for cocoa butter in chocolate and may represent an approach for developing more sustainable chocolate products in the future.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Edible insects and hidden risks: cross-reactive allergens in crustacean-allergic individuals

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Edible insects are increasingly promoted as sustainable alternative protein sources for food and feed applications. However, their taxonomic relatedness to crustaceans raises concerns regarding allergenic risk for individuals with shellfish allergy. While tropomyosin is a recognised pan-allergen, the diversity of clinically relevant insect allergens contributing to IgE cross-reactivity remains poorly characterised. This study characterises IgE reactivity to commercially available edible insects in a cohort of crustacean-allergic individuals, supported by proteomic and immunological analyses to inform allergen risk assessment. Protein extracts from commercial products derived from *Acheta domesticus* (cricket) and *Hermetia illucens* (black soldier fly; BSF) larvae were analysed by SDS-PAGE and immunoblotting. IgE-binding profiles were assessed using sera from 42 individuals with confirmed crustacean allergy. IgE-reactive proteins were identified by mass spectrometry and supported by whole-extract proteomic profiling. Cross-reactivity was further evaluated using allergen-specific antibodies raised against shrimp allergens (*Penaeus monodon* and *Penaeus vannamei*), alongside sequence conservation and putative IgE-epitope analyses. Patient serum IgE analysis identified both shared and species-specific insect allergens in cricket and BSF larvae, including tropomyosin, hemocyanin, myosin light chain, vitellogenin, heat shock proteins, apolipophorin, and chitin-binding proteins. Tropomyosin exhibited the highest IgE reactivity in cricket products (40%, n = 42). Supporting immunoblotting using shrimp allergen-specific antibodies demonstrated cross-reactive to shellfish allergens in cricket and BSF larvae. Proteomic profiling identified tropomyosin in cricket and hemocyanin in BSF larvae, with conserved allergen sequences showing >60% similarity across insect and crustacean homologues. These findings demonstrate that edible insects contain multiple IgE-reactive proteins beyond tropomyosin, with variable recognition among crustacean-allergic individuals. The presence of both shared and insect-specific allergens highlights the importance of targeted allergen profiling to support evidence-based risk assessment and the responsible integration of insect-derived proteins into food and feed systems.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Can enzymatic hydrolysis enhance the bioactivity and reduce allergenicity of *Tenebrio molitor* flour?

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Entomophagy is an ancient practice that has recently gained attention in Western countries as a sustainable alternative protein source. However, the inclusion of edible insects in the human diet raises safety concerns, particularly regarding their allergenic potential. The main concern is IgE cross-reactivity with crustacean protein allergens due to the close phylogenetic relationship between insects and crustaceans. Enzymatic hydrolysis is widely used in the food industry to improve safety and functionality and has been explored as a strategy to reduce food allergenicity. In this study, *T. molitor* larvae flour was hydrolysed by two endoproteases, namely alcalase (EC 3.4.21.62) and neutrase (EC 3.4.24.28), separately at concentrations ranging from 0.1% to 5% (w/w) under different temperatures and incubation times. The impact of hydrolysis on IgE cross-reactivity was evaluated using sera from crustacean-allergic patients by ELISA and immunoblotting. Additionally, the functional potential of the hydrolysates was assessed through inhibition of somatic angiotensin-converting enzyme (sACE; EC 3.4.15.1), associated with antihypertensive activity. The degree of hydrolysis was determined by quantification of free amino groups using the OPA/NAC assay. Enzymatic hydrolysis resulted in a significant reduction of IgE reactivity of insect flour after hydrolysis treatment with both enzymes. Moreover, hydrolysed samples showed significantly enhanced sACE inhibitory activity, with IC_{50} values indicating increased bioactivity (30.3 μg/ml for alcalase and 21.1 μg/ml for neutrase) compared to the non-hydrolysed larvae flour (690.1 μg/ml). *In vitro* gastrointestinal digestion (INFOGEST protocol) of selected hydrolysates (1% enzyme) demonstrated retention of bioactivity. These results support enzymatic hydrolysis as a promising approach to improve both the safety and functional value of insect-based ingredients for food applications. Funding: This work received financial support from European Union, FEDER, through the projects EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX), ALLEVIATE (2023.12193.PEX), UID/50006/2025, and IMMUNOGATE (COMPETE2030-FEDER-00848700).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Molecular detection of lesser mealworm (*Alphitobius diaperinus*) flour as an emerging allergen in processed foods

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The inclusion of insects as novel foods provides sustainable and nutritionally valuable alternatives to traditional protein sources. Among them, *Alphitobius diaperinus* (lesser mealworm) has recently been approved for human consumption under Regulation (EU) No 2015/2283, representing a significant milestone in the introduction of edible insects into the European Union (EU). However, introducing insects into the human diet raises safety concerns, especially regarding potential allergenicity, due to cross-reactivity in individuals allergic to other species like crustaceans and house dust mites. This work aims at developing a novel real-time PCR method for the detection and quantification of *A. diaperinus* larvae flour in food products. For this purpose, reference mixtures were prepared as model foods (wheat biscuit dough mixture) containing known amounts of *A. diaperinus* flour, ranging from 50 000 mg/kg to 0.1 mg/kg, subjected to thermal treatment to simulate processing. DNA extraction was performed using the NucleoSpin Food Kit, followed by UV spectrophotometry for DNA quantification. Following in silico studies and successful sequencing of genetic targets, species-specific primers and TaqMan probe were designed in the cytochrome c oxidase subunit I gene (COI) for the development of a real-time PCR method. The method demonstrated high specificity against 53 non-target species and achieved an absolute limit of detection (LOD) of 0.1 pg of insect DNA and a relative LOD of 1.0 mg/kg of insect flour in model food matrices. Calibration curves using processed reference mixtures met the performance criteria of PCR efficiency, correlation coefficient and slope, within a concentration range of 50 000 mg/kg to 0.1 mg/kg. The calibration model was effectively validated with blind mixtures, demonstrating the ability of the method to quantify *A. diaperinus* flour with acceptable accuracy and reproducibility parameters. Applicability of method was also demonstrated using 21 commercial samples of food and feed containing insects as an ingredient. This method provides a new, robust and reliable tool to detect *A. diaperinus* in processed foods, which can be useful to support insect traceability, food labelling compliance and allergen risk assessment, contributing to protect the health of sensitized individuals. Funding: This work received financial support from European Union, FEDER, through the project EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX) and UID/50006/2025.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

The influence mechanism of lactic acid bacteria to ensure the sustainability of insect food production

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The impact of lactic acid bacteria on quality insect food has been studied extensively. The study shows that the lactic acid bacteria are a diverse category of bacteria that produce lactic acid by digesting carbohydrates and widely used in a variety of industries, including the food and biomedical industries. This review will cover contemporary trends and issues in the use of lactic acid bacteria in the insect food and biomedical industries, particularly those that meet consumer demand in a safe manner. To ensure the sustainability of these microbial activities, it is important to understand the influence of lactic acid bacteria, the use of genetically manipulated microorganisms, separation techniques, different approaches to process organization have helped to solve challenges and possible solutions for process optimisation, the methods and characteristics of microbial fermentation have yielded positive results. Using these microbial strains, could derive the maximum benefit from its natural products and ensure the sustainability of insect food production.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Antioxidant and anti-inflammatory activity of waxworm and buffalo worm bioaccessible and bioavailable peptides

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Peptides derived from insect proteins are emerging as promising bioactive compounds for functional food applications. Previous research highlighted the nutritional and structural properties of insect proteins, specifically Buffalo worm (*Alphitobius diaperinus*) and Waxworm (*Galleria mellonella*). Comprehensive analyses of protein concentrates from both insects revealed their promising protein content and yields suitable for industrial production, exhibiting high *in vitro* protein digestibility (IVPD, 83.6%) and *in vitro* protein digestibility-corrected amino acid score (IVPD-CAAS, 62.7%). This study evaluated the antioxidant capacity, transepithelial absorption, and anti-inflammatory effects of peptides released during simulated gastrointestinal digestion of *Galleria mellonella* (Wworm) and *Alphitobius diaperinus* (Bworm) protein concentrates. INFOGEST digestates exhibited significantly higher antioxidant activity than gastric digestates, with enhanced reducing power (Fe^{2+}, Cu^{2+}) and radical scavenging capacity (ABTS, DPPH). Intracellular reactive oxygen species (ROS) inhibition reached 40.2% for Wworm and 58.5% for Bworm. Transepithelial transport using Caco-2 monolayers showed that peptide absorption mainly occurred between 6 and 24 h, with Wworm presenting a higher apparent permeability coefficient (6.10×10^{-6} cm^2/s) than Bworm (5.91×10^{-7} cm^2/s). Peptides were identified by LC-MS/MS and subjected to in silico anti-inflammatory prediction and molecular docking, highlighting interactions with IKK-β, a key regulator of the NF-κB pathway. At concentrations up to 500 μg protein/ml, intestinal digestates reduced macrophage inflammatory signalling by 29% in Wworm and 54% in Bworm. Bworm digestates showed greater nitric oxide reduction (13.2–15.8%), while both samples significantly downregulated pro-inflammatory cytokines (IL-6, TNF-α, IFN-γ, IL-1β) by 18.8–74%. This response was accompanied by a 15-fold increase in HO-1 mRNA expression, suggesting activation of the Nrf2/HO-1 pathway. Digestion-derived peptides from both insect species exhibit combined antioxidant, absorptive, and anti-inflammatory properties, supporting their potential as multifunctional bioactive ingredients in alternative protein systems.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Enhancing recipe healthfulness by replacing conventional meats with edible insects

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The cost of conventional meat recipes e.g., beef, poultry, pork, and fish is rising thus creating demand for more affordable, but healthful alternatives. Edible insects have been suggested as suitable sources of valuable nutrients for humans and could therefore be substitutes for conventional meats in recipes. It is not yet clear if replacing conventional meats with edible insects would have a positive or negative effect on the nutritional profiles and the healthfulness of diets. Conventional meat recipes nutrient data were obtained from Kenyan Food Recipes 2018 book and Malawi Food Composition Table 2019. The nutritional data of the recipes were retrieved from the respective Food Composition Tables (FCT) of Kenya and Malawi. Cricket, termite, and grasshopper were selected to replace the meat items in the conventional meat recipes. Nutritional data of edible insects was obtained from a systematic review. Nutrient profile scores for the recipes were generated to get the healthfulness by three nutrient profiling models: WXYfm, RRR, and GDA. A two samples T-test (Tukey's Studentized Range (HSD) Test (The SAS System)) was performed to compare the healthfulness scores (WXYfm, RRR and GDA) of conventional meat recipes and edible insects-substitute recipes between Kenya and Malawi while a paired *t*-test compared data from within each country. A total of 21 conventional meat recipes were retrieved from the Kenyan FCT and 13 were obtained from Malawian FCT. According to WXYfm, 35% of the conventional meat recipes from Kenya would be classified as less healthful compared to 0% of the Malawian recipes, and substitution of the meat items with edible insects did not adversely affect the classification. All Malawian meat-based recipes were healthful, while 52.4% of the Kenyan recipes would be classified as unhealthful according to RRR model, but substitution with edible insects improved the scores. Based on GDA, all the edible insects-substitute recipes were significantly ($p < 0.05$) different from the conventional meat recipes, which indicated that substitution had a positive impact on the recipes' healthfulness. More healthful diets are associated with better health outcomes in the population. Manufactured food products that incorporate cricket flour as an ingredient, e.g., cricket protein bars, are already available in the USA market. Thus, inclusion of edible insects in daily diets would be in alignment with the current food industry trends. Studies on the acceptability of edible insects as human food in daily diets need to be carried out to improve on the available data.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Impact of diet on *Tenebrio molitor* oil: composition, oxidative stability and digestibility

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While insect research has traditionally focused on proteins, insect lipids are gaining attention as nutritive and sustainable lipid sources. *Tenebrio molitor* larvae are rich in unsaturated lipids, dominated by oleic and linoleic acids. Although larval diet affects lipid composition, evidence of the oxidative stability and digestibility of *T. molitor* oil (TMO) remains limited. Thus, this study aimed to evaluate the effect of larval diet based on popular by-products from olive oil industry (olive leaves, OL; olive pomace, OP), or quinoa crops (quinoa husk, QH) on the composition, oxidative stability, and digestibility of TMO. TM larvae were reared in triplicate (15 000 larvae; 4–6 weeks-old; 4.8 mg/larva; 400 mg feed/larva) for 7 weeks (26 °C, 55% RH, water sprayed) on wheat bran as control (C) or partially replaced with OL (15, 30 and 50%), OP (30, 50, 70 and 90%) or QH (15, 30 and 50%). Reared larvae were blanched (100 °C, 1 min), oven-dried (60 °C, 18 h), and defatted by supercritical CO_2 (450 bar, 60 °C, 100 g min^{-1}) to obtain the lipid fraction. Fatty acid composition (GC–MS) was analysed, and oxidative stability monitored for 28 weeks (peroxide value, PV). *In vitro* digestibility was assessed by simulated intestinal digestion and compared with olive and soybean oils. Despite some slight modifications due to the diets, the observed fatty acid profiles were consistent with those expected for TMO. Nevertheless, larvae fed OP tended to lower SFA, higher MUFA and lower PUFA contents. Oxidative stability was affected by diet. Thus, oils from larvae fed all levels of QH, OL30, OP30 and OP70 showed higher oxidative stability (PV<10 mEq O_2/kg), compared to C-TMO (>15 mEq O_2/kg) after 7 months of storage. Digestion showed slightly higher lipid hydrolysis across all treatments (62-66% of free fatty acids), compared to olive (58%) and soybean (60%) oils, and high bioaccessibility (>70%), indicating high digestibility, comparable to vegetable oils, and regardless of diets. Insect diet is an effective strategy to modulate the lipid composition and improve the oxidative quality of *T. molitor* oils, without compromising its high digestibility, supporting its potential as a sustainable alternative lipid source for food and feed, while simultaneously valorising agri-food by-products. Acknowledgement: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Characterisation of supercritical CO_2-extracted omega-3-enriched oil from *Tenebrio molitor* larvae

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Insect flours are an emerging sustainable nutrient source with promising lipid profiles for value-added functional ingredients. In this study, lipid fractions were obtained from *Tenebrio molitor* larvae by supercritical CO_2 extraction under different operating conditions, with the objective of producing an omega-3-enriched functional oil. To enhance the omega-3 content of the lipid fraction, the larvae were reared on a diet supplemented with flaxseed cake. The extracted lipids were characterised in terms of basic quality parameters, fatty acid composition, FTIR profiles, and bioactive components (tocopherols and total phenolic content). The extracted oils exhibited moisture contents between 5.9 and 24.4%, peroxide values ranging from 0.68 to 3.10 mmol/kg oil, and acid values between 7.3 and 41.9 mg KOH/g oil. The dominant fatty acid was the MUFA oleic acid (ω-9), accounting for 47.7–50.2% of the total fatty acid content. It was followed by the PUFA linoleic acid (C18:2n6c), present in the 20.4–20.8% range, along with the SFAs palmitic acid (C16:0) and myristic acid (C14:0), which ranged from 15.8 to 17.1% and 4.5–5.9%, respectively. Alpha-linolenic acid (ω-3), a nutritionally relevant PUFA, was detected at levels of 3.0–4.2%, resulting in an ω-6/ω-3 ratio of 4.9–6.9, indicative of an improved fatty acid balance. FTIR analysis showed consistent spectral profiles across all extracts, closely matching those of olive oil and confirming a predominantly triglyceride structure typical of natural edible oils. The extracted oil contained appreciable levels of tocopherols, with total contents between 72.2 and 130.3 mg/kg oil, dominated by α-tocopherol, while total phenolic content (TPC) ranged from 18.9 to 187.5 mg GAE/kg oil. Overall, the composition and bioactive components of the extracted oil suggest its potential for future use in food and cosmetic applications, particularly after suitable post-extraction processing. Acknowledgements: This work was financed by Ministry of Science, Technological Development, and Innovations of the Republic of Serbia (contracts nos. 451-03-136/2025-03/200222, 451-03-137/2025-03/200134, and 451-03-137/2025-03/ 200117).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Optimised recovery of oil from *Encosternum delegorguei* for food and nutraceutical applications

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This study optimised the extraction and characterisation of oil from edible stink bugs (*Encosternum delegorguei*) using Response Surface Methodology (RSM) with a Central Composite Design (CCD), varying binary solvent systems (ethanol:chloroform, ethanol:hexane, ethanol:petroleum ether), extraction time (3–5.6 h), and temperature (40–64 °C). Optimal conditions yielded over 54%, with the highest yield (56.46 ± 0.03%) obtained using ethanol:petroleum ether at 57.03 °C for 5 h. Oils demonstrated favourable nutritional quality, with low atherogenic (0.62–1.17) and thrombogenic indices (0.66–1.07), and high hypocholesterolemic/hypercholesterolemic ratios (up to 4.83), indicating cardioprotective potential. Stability indices such as iodine value, acid value, and saponification value varied with saturation degree and extraction conditions, suggesting good oxidative stability under optimised parameters. These results support the potential of *E. delegorguei* oil as a sustainable ingredient for functional foods, plant-based spreads, nutraceutical capsules and fortified food products.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Tracing *Bombyx mori* as a potential adulterant in food and feed

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Edible insects are gaining attention as a sustainable and nutritious alternative to animal protein. To date, the European Union (EU) has only authorised the placing on the market of 4 insect species under the novel food Regulation (EU) No 2015/2283, namely: *Tenebrio molitor* larva, *Locusta migratoria*, *Acheta domesticus* and *Alphitobius diaperinus* larva. Since the availability of foods containing insects remains limited, the price of these 4 insect species is comparatively high due to a combination of factors, including the reduced number of authorised producers and the limited quantities of produced insects. This has led to a growing use of non-regulated insects as adulterants in EU food products. One such adulterant species is the *Bombyx mori* (silkworm), an insect intensively used for silk production and traditionally consumed as a food in Asian countries. As a by-product of silk manufacture, it is inexpensive, readily available, and easy to acquire. Therefore, the availability of tools to detect *B. mori* as a potential adulterant in foods is a key safety issue. The present work intends the development of novel molecular tools for quantitative and rapid detection of *B. mori* in foods. To this end, reference model mixtures of pancakes containing 20% (w/w) of insect flour (*T. molitor* + *B. mori*) with known proportions of *B. mori* (0.1% to 100% of *B. mori* pupae in *T. molitor* larvae) were prepared. DNA was extracted using the NucleoSpin Food kit. Several primers targeting nuclear and mitochondrial DNA were designed and tested both in silico and *in vitro* for specificity. The results of primer testing by qualitative PCR confirmed the specificity of primers for the target species using several non-target insect, animal and plant species used as food. A real-time PCR assay with a TaqMan probe was developed with the best performing primers targeting cytochrome oxidase subunit III gene of *B. mori*, showing high sensitivity (10 pg of DNA) and suitable performance parameters of PCR efficiency, slope and correlation within 0.1–100% of *B. mori* in *T. molitor*. The method was successfully validated and applied to marketed food and feedstuffs. In parallel, a loop-mediated isothermal amplification (LAMP) method is being developed as a simple, rapid, cost-effective and point-of-care tool to be easily applied by the food industry, without requiring sophisticated equipment. Funding: This work received financial support from European Union, FEDER, through the project EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX) and UID/50006/2025.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

From the hive to the table: safety and nutritional characterisation of honey bee larvae and pupae flours

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The Western honey bee (*Apis mellifera* L.) is the most widely managed insect species worldwide. Recently, increasing interests in insects as alternative food sources has expanded to include products derived from this species, such as powders produced from larvae and pupae. Unlike other edible insects, honey bees are not reared under industrial or mass-production conditions. Instead, they are managed within apiaries and have free access to the environment, where they autonomously forage for food and water. This aspect increases exposure to pathogens and contaminants, raising concerns for the safety and quality of food products derived from this species. In parallel, there is a concerning scarcity of data on the nutritional value, microbiological safety, and contaminant profile of honey bee–derived novel foods. We here provide a comprehensive characterisation of two novel foods commercialised in East Asia: honey bee queen larvae flour and drone pupae flour. One sample of each product was collected and subjected to centesimal analysis to determine macronutrient composition. In parallel, microbiological investigations were used to assess the presence of relevant bee pathogens and food safety indicators. Samples were also screened for heavy metals and a broad range of contaminants, including pesticide and acaricide residues. Analyses revealed that the two samples presented distinct macronutrient composition, reflecting their different casts. Spores of *Clostridium botulinum* and *Paenibacillus larvae* (the causative agent of American foulbrood in honey bee larvae) were absent in both samples. Most chemical contaminants were below the limit of quantification (LOQ, 0.010 mg/kg), except for Carbendazim (0.295 mg/kg), a fungicide currently banned in the European Union, which was found in drone bee powder. Heavy metals were detected at low levels: arsenic was found in larvae powder (0.020 ± 0.003 mg/kg), nickel was detected in both matrices (0.07 ± 0.02 mg/kg in pupae and 0.24 ± 0.05 mg/kg in larvae) while lead, cadmium, and mercury were below the LOQ. Overall, this study provides one of the few integrated evaluations available on the nutritional quality, microbiological safety, and contaminant profile of honey bee–derived flours. Considering the growing interest towards the production of honey bee-derived novel foods, our results underline the need for tailored controls and monitoring of such products to ensure consistent food safety and quality standards.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Development of a cricket-flour protein beverage: a sustainable and nutritionally viable alternative

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Growing population pressure and environmental constraints are accelerating the search for sustainable protein sources. Edible insects are emerging as a nutritionally robust and resource-efficient alternative, addressing land and water use associated with livestock production. This work aimed to develop a dairy-based protein beverage using cricket flour (CF) as an alternative protein source aligned with health and sustainability trends. A multi-stage product development strategy was implemented to formulate CF beverages in ready-to-drink (RTD) and powdered formats. For RTD variants, dairy-based prototypes were developed across five flavour matrices and initially assessed at three CF inclusion levels (1, 5 and 10% w/w). In the second stage, powdered concepts were developed due to lower perishability and improved logistical efficiency compared to RTD formulations. Powdered formulations included 2.5 and 5% (w/w) CF combined with three flavour matrices. Across all prototypes, ingredient proportions were adjusted to ensure that 20% of the product's energy value was derived from protein. Physicochemical characterisation supported optimisation, focusing on key quality and stability parameters (e.g., pH, colour, density). During initial screening, 10% (w/w) RTD formulations were discontinued due to pronounced thickening, creating clear technological constraints. Additionally, three flavour matrices were excluded due to limited compatibility with the dairy system, namely progressive thickening during short-term storage and colour changes affecting product quality. Following iterative optimisation supported by physicochemical profiling, development converged on one promising RTD prototype containing 1% (w/w) CF, which showed the most stable performance. In powdered format, three promising prototypes were obtained, incorporating 2.5% (w/w) CF in two flavour matrices and 5% (w/w) CF in a third matrix. This work confirms the feasibility of developing dairy-based protein beverages enriched with CF in RTD and powdered formats. Performance depended on inclusion level and flavour matrix, with clear limits related to thickening and appearance. Overall, powdered formulations appear the most practical and scalable route to deliver sustainable, nutritionally relevant insect-derived protein. This work was funded by InsectERA (No. C644917393-00000032), WP InFood, supported by Next Generation EU and Portugal's PRR under "Agendas para a Inovação Empresarial" (C5).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Tenebrio molitor larvae as a sustainable alternative for developing nutritious beverages

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As global demand for sustainable and alternative protein sources continues to rise, edible insects have garnered significant attention as ingredients for developing beverages. In this study, *Tenebrio molitor* (TM) larvae were used to produce oil-in-water (O/W) emulsions mimicking a dairy milk analog. TM larvae were starved (48 hours) and thermally treated. Subsequently, chitin-free TM slurry was prepared using a sodium phosphate buffer (pH 7) and stabilized with gellan gum (0.015%, w/w) and lecithin (0.15%, w/w). Four milk analog formulations were developed by adding rapeseed oil at 0% (Control), 2, 3 and 4% levels, followed by ultra homogenization at 10 000 rpm and storage at 4 °C for 10 days. The results demonstrated a milk-like consistency with a protein content of 1.20%, which was higher than that of some commercial vegan options, such as oats and cashews based-beverages. The lipid content varied from 0.9 to 4.6% in accordance with the level of oil addition. The phosphate buffer system effectively maintained pH levels between 7.03 and 7.08 throughout the storage period, indicating high chemical stability. The median droplet size decreased from 12.67 μm in the control to 4.08 μm in the 2% oil formulation. Increasing the oil content raised the Whiteness Index (WI) from 53.95 to 65.49 and improved emulsion stability, as evidenced by a reduction in the creaming rate from 0.48 to 0.36 compared with the control samples. In conclusion, *Tenebrio molitor*-based milk analogs are a viable option for developing nutrient-dense and sustainable dairy alternatives.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Induction of systemic antimicrobial activity in *Hermetia illucens* larvae following exposure to diatomaceous earth powder

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The rapid growth of the global population is driving an expansion in livestock production, intensifying the demand for sustainable, nutritionally efficient feed resources. At the same time the misuse of antibiotics in animal agriculture has accelerated antibiotic resistance, threatened animal and public health, and underscored the need for effective alternatives. In this context, insects, particularly *Hermetia illucens* larvae (black soldier fly larvae, BSFL), have emerged as promising nutrient-dense feed ingredients. Furthermore, insect are a rich source of antimicrobial peptides (AMPs), which exhibit broad-spectrum activity and a low risk of inducing bacterial resistance. AMPs are increasingly recognized as promising substitutes for traditional antibiotics in livestock feed. However, a key challenge is identifying rearing conditions that enhance the systemic production of AMPs in BSFL. One potential strategy involves the administration of feed additives able to stimulate the larval humoral immune response and promote AMP production in the haemolymph. Diatomaceous earth powder (DE), a mineral-based insecticide causing physical damage to insects, has been used for this purpose. We characterised the systemic humoral immune response of BSFL following oral exposure to different concentrations of DE, either alone or in combination with Gram-positive (G+) and Gram-negative (G–) bacteria, in both live and heat-killed forms. Larval responses were assessed by monitoring survival and timing of adult eclosion. Haemolymph AMP production was evaluated three days post-exposure using microbiological and biochemical assays. Results were further validated by qPCR analysis of representative AMP genes. We found no significant effects of DE oral exposure on vitality, although transient signs of sickness were observed. Additionally, haemolymph of larvae treated with 20% DE exhibited notable antibacterial activity against both G+ and G– bacteria compared to controls and other treatment groups. Although AMP mRNA levels were not significantly altered, larvae exposed to 20% DE showed a consistent trend toward increased expression, indicating that this condition may be optimal for generating AMP-enriched BSFLs to be used as feed additives.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Live black soldier fly larvae in the diet of suckling piglets increases their pre-weaning voluntary feed intake

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Piglets naturally adapt to solid feedstuff (such as plants, nuts, and insects) over several months. Commercially reared piglets are usually weaned abruptly at four weeks of age, despite having little experience with solid feed, which can result in prolonged post-weaning feed refusal and long-term health and performance impairments. The aim of this study was to accustom piglets to solid feed as early as possible to adjust their digestion ("enzyme training") for the post-weaning period. One potential strategy is supplementing live black soldier fly larvae (LBSFL), which are low in dry matter and carbohydrates (CHO) but rich in protein and fat. The low CHO content of BSFL might facilitate easier digestive adaptation, as fewer CHO-degrading enzymes are required. Therefore, BSFL may represent a suitable feedstuff for piglets during the transition from liquid milk to solid diets. In this study, a total of 599 piglets (average initial body weight of 1.58 kg and age of 1.5 days) from 43 sows (average of 14 piglets/litter) across two experimental runs were equally allocated into two treatments: CON = creep feed (CRF), LAR = CRF + LBSFL. Piglets were weighed at trial start and weekly, on days 7, 14, 21 and 28, to evaluate growth performance. LBSFL and CRF were offered in separate troughs to record feed intake. Each litter had access to two troughs (CON: 2 × CRF, LAR: 1 × CRF + 1 × LBSFL) to ensure equal feeding space availability. CRF and LBSFL were weighed daily and offered fresh daily. If a feed trough was empty the next day, the amount of CRF or LBSFL was increased to simulate *ad libitum* feeding. Data were analysed using one-way-ANOVA (SPSS, IBM). LBSFL feeding did not affect the weaning weight of the piglets on day 28 ($P > 0.05$). Piglets fed LBSFL tended to have a 32% higher average daily CRF intake compared to CON during the entire suckling period ($P = 0.057$), while CRF intake was 42% higher in the last week before weaning ($P = 0.022$). In addition, LBSFL feeding resulted in a 77% increase in total DM intake (CRF incl. LBSFL on DM basis) during the entire suckling period ($P < 0.001$), compared to CON. This led to an increase in overall nutrient intake, especially lysine (+56%, $P < 0.001$) and metabolizable energy (+91%, $P < 0.001$). In conclusion, LBSFL feeding promotes overall voluntary feed intake during the suckling period and enables the uptake of a diverse range of feedstuffs.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutrient digestibility of feedstuffs derived from *Tenebrio molitor* in pigs

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In pig nutrition, insect-derived feedstuffs are of interest due to their sustainability, use in circular economy and many other aspects (van Huis *et al*., 2021). Calculating diets according to pig's nutrient requirements requires data on nutritive value of these feeds. Thus, we determined nutrient digestibility of *Tenebrio molitor* larvae (TML) feedstuffs from different feed processing methods. TML were reared in one production site at identical conditions, slaughtered and processed into: full-fat TML (dried and ground), defatted TML protein meal (TML-PM) and defatted, chitin-reduced TML protein meal (TML-CHIT). A trial was conducted with eight growing barrows, surgically equipped with a simple T-canula at the terminal ileum (Society of Nutrition Physiology, 2005). Four experimental diets (incl. indigestible marker), i.e. basal diet BD and three assay diets with TML, TML-PM and TML-CHIT (each at 30% inclusion), were tested simultaneously using the difference method, with 2 pigs/diet in a Latin square design (n = 8/feedstuff, 4 periods). Daily feed allowance was calculated based on metabolic body weight (Schiemann, 1981), any feed refusal collected and individual feed intake determined. Ileal digesta and faeces were collected per pig and period, freeze-dried, homogenized and analysed. Apparent ileal and total tract digestibility coefficients ($DC_{ileal/total}$) were calculated for each feedstuff using the indigestible marker technique[2]. Chitin analysis showed the successful technological reduction of chitin in the product TML-CHIT (3.0% DM) as compared to TML-PM (12.2% DM) and TML (8.9% DM). Digestibility of organic matter did not differ between feedstuffs (DC_{total} in % = 83 ± 2; DC_{ileal} = 69 ± 1). For nitrogen, we observed a distinctly lower DC_{ileal} for TML (in %: TML = 76, TML-PM = 81, TML-CHIT = 80) and the highest DC_{total} for TML-CHIT (in %: TML = 88, TML-PM = 89, TML-CHIT = 92). Chitin degradation took place primarily in the large intestine as expected, ranging from 43% in TML-CHIT to 70% in TML-PM. Surprisingly, we did see a rather substantial chitin degradation at an ileal level for TML-PM (40% DC_{ileal}), but none for the other two feedstuffs, hinting at a different enzymatic accessibility of this fraction due to feed processing. Acknowldgement: This research was part of Joint Research Project TeMoTech. References: Schiemann, R., 1981. Methodische Richtlinien zur Surchführung von Verdauungsversuchen für die Futterwertschätzung. Archives of Animal Nutrition 31: 1-19. Society of Nutrition Physiology, 2005. Proceedings of the Society of Nutrition Physiology 14: 185-213. van Huis, A., Rumpold, B.A., van der Fels-Klerx, H.J. and Tomberlin, J.K., 2021. Advancing edible insects as food and feed in a circular economy. Journal of Insects as Food and Feed 935-948.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Black soldier fly oil as a lipid source in guinea pig diets: effects on growth, health markers, and consumer acceptance

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The production of sustainable animal-source foods is increasingly constrained by competition for conventional lipid feed resources from crops and fisheries. Insect-derived oils offer a promising alternative, yet evidence on their effects on animal performance, physiological responses, product quality, and consumer acceptance remains limited. In this study, we formulated four diets by replacing soybean oil (SBO) with black soldier fly (*Hermetia illucens* L.) oil (BSFO) on an equal-lipid basis: SBO100 (0% BSFO), BSFO50, BSFO75 and BSFO100 (100% BSFO), and evaluated them in a controlled feeding trial with guinea pigs (*Cavia porcellus* L.). Feed intake, growth performance, feed efficiency, physiological biomarkers, and consumer acceptance of meat from BSFO-fed guinea pigs were assessed. Guinea pigs readily accepted BSF oil-based diets, with no mortality observed. Feed intake differed by sex and showed a significant diet × sex interaction, whereas average daily gain and feed conversion ratio were unaffected by diet or sex. Diet-specific physiological patterns were evident, with BSFO50 achieving the highest overall composite score (0.23) and BSFO100 markedly enhancing erythron/oxygen-transport indices (0.52). BSF oil substitution induced organ-specific effects, increasing heart weight at moderate inclusion (BSFO50) and reducing lung and spleen weights at higher inclusion levels, without affecting liver, kidney, or intestinal mass. Replacing soybean oil with BSF oil did not negatively affect meat taste or consumer acceptance. Willingness to buy was influenced by age and taste ($p = 0.0087$), but not by diet ($p = 0.48$), with over 75% of older consumers showing the highest acceptance, with flavour outweighing sustainability or novelty as the primary driver. Overall, BSF oil is a promising lipid source in guinea pig diets. Its application in feed supports normal growth, animal health, and consumer acceptance and willingness to purchase guinea pig meat. These findings highlight the potential of insect-derived oils to enhance the sustainability of small livestock production systems.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Effects of black soldier fly larvae as dietary protein source on growth performance and gut health in weaned piglets

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Black soldier fly larvae (BSFL) meal is a promising sustainable protein source for livestock feed. The effects were evaluated of diets containing a commercial batch of BSFL meal with assessed nutrient digestibility on growth performance and intestinal health of weaned piglets. A total of 324 weaned male piglets (27 days of age; mean body weight 7.7 kg) were assigned to three dietary treatments: a control diet, and diets containing 5% or 10% defatted BSFL meal, each nutritionally balanced. Diets were provided in pre-starter (day 1–11) and starter (day 11–34) phases. Piglets were housed in pens with nine animals (n = 12 pens/treatment) and fed *ad libitum*. Growth performance, health indicators (veterinary treatments, weekly faecal consistency, skin and tail lesions in week 1 and 4 after weaning), and serum acute phase proteins, pro-inflammatory cytokines, and jejunal morphology were determined at day 34. Data were analysed using ANOVA with pen as the experimental unit. Body weight at day 34, daily feed intake and body weight gain, and feed conversion ratio (FCR) did not differ significantly among treatments. Only during the pre-starter phase (day 1–11), FCR was slightly higher ($P = 0.048$) in piglets fed 10% BSFL compared with the control. Frequency of veterinary treatments, faecal consistency, and incidence of skin or tail lesions were not affected by BSFL inclusion. Concentrations of serum acute phase proteins and cytokines were similar among treatments. Jejunal villus height, crypt depth, and villus-to-crypt ratio were unaffected by BSFL inclusion. Inclusion of up to 10% defatted BSFL meal diets ensured good growth performance and health status of weaned piglets reared under high sanitary conditions. The lack of differences in inflammatory markers and gut morphology suggests that BSFL protein is well tolerated and does not challenge gut integrity. In conclusion, defatted BSFL meal with verified nutrient digestibility can be included up to 10% in post-weaning piglet diets without effects on growth performance, intestinal morphology, or systemic health, supporting its potential as a sustainable alternative protein source in pig nutrition.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Comprehensive evaluation of meat quality in pigs manure fed housefly (*Musca domestica*) larvae reared on swine manure

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The present study investigated the use of houseflies reared on faeces from specific pathogen-free (SPF) pigs as a feed source for pigs, focusing on its impact on pork quality. In accordance with Japanese guidelines for insect production, the researchers collected housefly larvae (*Musca domestica*) and incorporated them into pig feed, with 5% of the feed composed of this insect protein. The experiment involved the testing of two distinct groups of pigs: weaned piglets and finishing pigs. The control group was fed a standard diet, while the test group was fed an insect-based diet. Subsequent to slaughter, longissimus dorsi muscle samples were analysed for fibre structure, amino acid profiles, and fatty acid composition, in conjunction with sensory evaluations of taste and quality. The findings indicated that pigs fed housefly larvae exhibited greater weight gain than the control group and received favourable ratings for palatability compared with lean meat. The analysis of muscle tissue revealed that the subjects of the experiment, pigs fed an insect diet, exhibited favourable morphology associated with improved water retention. In addition, the levels of umami-related amino acids in the subjects were elevated. Further research is underway to ascertain alterations in collagen amino acids and fatty acid distribution within the meat. This study demonstrates the potential of utilising insect-derived feed from organic waste to enhance the nutritional quality and flavour of pork, thereby providing a sustainable solution within the swine industry.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Can we replace soya oil in broiler diets with *Tenebrio molitor* larvae oil?

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Insects are a natural feedstuff for poultry, particularly in free-ranging chicken consumption of insect species are common. Besides the often-reported use as protein source, insects also contain high amounts of fat that might be used as a valuable energy source. In poultry, insects such as *Tenebrio molitor* larvae (TML), could be a promising, regionally available source of protein and fat in broiler diets. The aim of this study was to determine the effects of gradually replacing dietary soybean oil with TML-fat on performance parameters in fast growing broilers. TML were reared in one production site at identical conditions, slaughtered and processed (fat mechanically separated). Fat was provided to 288 fast growing broilers (Ross 308) during the entire 38 days-lasting fattening period. The day-old chicks were purchased from a commercial hatchery and housed in 24 pens, each containing 12 animals. In addition to a soybean meal/wheat/corn-based control diet containing only soybean oil, four other experimental diets were designed in which only the soybean oil was replaced at 25, 50, 75 and 100% with TML-fat. Feed consumption per pen and body mass of the animals were recorded weekly. On days 36–38 of life, four animals per pen were killed per day in order to collect various tissues (internal organs and digesta) for further analyses. The live weight at the end of the fattening period was not affected by the change in dietary fat source ($p = 0.438$). Viscosity in small intestinal digesta did not show dose-response-related changes, but some variations between feeding groups. The pH-values in caecal content were not affected by the experimental diets ($p = 0.391$). In summary, first evaluations did not identify any significant effects of replacing dietary soybean oil with TML-fat on broiler performance. Further evaluations are ongoing as part of the TeMoTech joint project, which aim to provide deeper insight into the effects of TML products in poultry nutrition. This research was part of Joint Research Project TeMoTech.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

How do live larvae modulate reproductive performance, welfare, and behavior of dual-purpose chicken breeders?

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Benefits of using live larvae in poultry nutrition on bird production and welfare were observed in the past decade in commercial egg and meat-hybrids, and slow growing chickens. However, no information is available on the use of live larvae in chicken breeder's diet. This study investigated the effect of 0% (CON), 5% (TM5) and 10% (TM10) diet-based inclusion of live yellow mealworms (*Tenebrio molitor*) on reproductive performance (fertility, hatchability, stages of embryo mortality), behavior (mating and courtship characteristics), ethological tests (familiar and stranger human approach test–HAF, HAS, outdoor access tests–UAT), and physical and physiological parameters (feather coverage, corticosterone and testosterone) of 40-week-old native dual-purpose chickens for 84 days (beginning (T0), middle (T1) and end of the trial (T2)). Data were analysed through Generalized Estimating Equations and Cox regressions and pairwise comparisons were adjusted by Bonferroni correction (IBM SPSS v20.0, $P \leq 0.05$). Fertility was greater at T2 than T0 but in TM5 groups only ($P < 0.01$). Moreover, a greater proportion of TM10 birds mated around live larvae distribution when compared to TM5 at T2 ($P < 0.05$). Mating duration at T0 in CON was lower than TM5 ($P = 0.001$) but did not differ from TM10. Finally, at T2 TM10 displayed the shortest mating duration (vs CON ($P = 0.001$) and TM5 ($P < 0.05$)), while CON groups the longest (vs TM5 ($P < 0.01$) and TM10 ($P = 0.001$)). A greater proportion of birds approached the operator in the HAF in TM5 and TM10 when compared to CON groups ($P < 0.001$), despite no differences being observed between TM5 and TM10 ($P > 0.05$). Roosters in TM5 showed a lower latency of outdoor area access than CON ($P < 0.01$) and TM10 ($P < 0.001$), while no differences were observed between TM5 and TM10 birds ($P > 0.05$). Finally, the latency of outdoor access for roosters at T2 was greater than at T0 ($P < 0.01$). Feather coverage and corticosterone were unaffected by diet ($P > 0.05$). A greater proportion of birds was observed in TM10 when compared to TM5 during the HAS ($P < 0.05$). Finally, testosterone concentration (ng/mg) was greater in TM10 than CON at T2 ($P < 0.001$), as well as when comparing T2 to T0 in TM10 birds ($P < 0.001$). The current findings indicate that the administration of live larvae has the potential to affect dual-purpose chicken breeders' reproductive performance and behaviour, and its use may be investigated to address welfare issues in farms. Acknowledgement: Research supported by the EU-PRIMA project ADVAGROMED (PRIMA, 2021–section 2).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Dietary black soldier fly larvae meal alters serum metabolomic profiles in Silkie chicken

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Black soldier fly larvae (BSFL; *Hermetia illucens*) meal, an alternative protein source for poultry diets, is rich in medium-chain fatty acids and bioactive compounds which can influence metabolism and gut health. This study aimed to investigate mechanisms underlying the observed improvements in growth performance by assessing serum metabolome and gut health indicators in slow-growing Silkie crossbreed chickens. Birds were fed a soybean-based control diet or a diet containing 150 g/kg partially defatted BSFL meal from 39 to 60 days of age. Growth performance parameters and serum concentrations of lipopolysaccharides (LPS), D-lactate, and alkaline phosphatase (ALP) were measured. At the end of the trial, serum was analysed using untargeted liquid chromatography–mass spectrometry-based metabolomics. BSFL inclusion significantly increased average daily gain over the trial ($p = 0.02$) and during week 2 ($p = 0.01$), while feed conversion ratio was numerically lower ($p = 0.08$) without affecting feed intake. Serum D-lactate was significantly reduced ($p < 0.01$) and LPS showed a decreasing trend ($p = 0.09$), whereas ALP was unchanged. Metabolomics detected 3,304 serum features, of which 777 annotated metabolites were retained. PCA and PERMANOVA demonstrated clear separation between dietary groups ($p = 0.001$). Forty metabolites differed significantly (FDR≤0.05), with 31 increased and 9 decreased in BSFL-fed chickens. Differential metabolites were dominated by lipid-related compounds, including medium-chain fatty acids, phosphatidylcholines, lysophosphatidylcholines and sphingomyelins, reflecting altered lipid metabolism and membrane remodelling. Pathway enrichment indicated fatty acid biosynthesis and mitochondrial β-oxidation of medium-chain fatty acids and very-long-chain fatty acids ($p < 0.001$). Microbial-associated metabolites and amino acid derivatives were also affected, suggesting potential interactions between diet, gut microbial activity, and host metabolism. Several lipid species were positively correlated with daily gain and negatively correlated with feed conversion ratio and D-lactate. These findings link BSFL-associated changes in the serum metabolome with improved growth performance and gut health indicators, highlighting candidate metabolic pathways for future mechanistic validation.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Effects of yellow mealworm meal and oilseed cakes on growth performance and carcass traits of broiler chickens

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The poultry industry is exploring alternative protein sources to enhance sustainability and reduce reliance on soybean meal. This study investigated the effects of yellow mealworm (*Tenebrio molitor*; TM) meal and oilseed cakes from linseed (*Linum usitatissimum*) and camelina (*Camelina sativa*) on broiler performance and carcass traits. Two hundred and forty male Ross 308 broiler chicks were randomly allocated to three isoenergy and isonitrogenous (metabolizable energy: 3237 kcal/kg; crude protein: 19.2% as fed) dietary treatments: control (10% soybean meal), insect (10% TM meal), and cake (5% linseed cake + 5% camelina cake). Chicks were housed in 12 pens (4 replicates/treatment; 20 birds/pen; 3.25 m² surface) under controlled conditions. Birds received *ad libitum* access to commercial diets (starter: 1–7 days, grower: 8–21 days) then to experimental finisher diets (21–42 days). Individual body weights and pen feed intake were recorded weekly. At 42 days, all birds were processed at a commercial slaughterhouse following standard procedures. A total of 45 carcasses (15 per treatment) were collected for carcass traits and meat physical analysis (pHu, L^*, a^*, b^* colour values, thawing and cooking losses, and shear force). One-way ANOVA (SAS Institute, Cary, NC, USA) was used to analyse data. Results observed during the trial period (21–42 days) showed no significant differences in growth performance and feed intake among treatments. However, the insect diet resulted in the most efficient FCR compared to the cake diet (1.81 vs 1.94, respectively; $P < 0.01$) with intermediate FCR for the control diet (1.88). Carcass traits and meat physical properties were not affected by the experimental diets. This study demonstrates that partial replacement of soybean meal with TM meal (up to the 10% inclusion level) in finisher broiler diets can maintain growth performance and potentially improve feed efficiency. The dietary inclusion of the two oil cakes at 5% level each are irrelevant for the considered traits. In conclusion, this research provides valuable information for feed manufacturers and poultry producers considering the incorporation of insect meal or oilseed by-products, in broiler diets. The study contributes to the growing body of evidence supporting the use of alternative protein sources in sustainable poultry production, offering potential pathways to reduce environmental impact while maintaining or improving production efficiency.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutraceutical-enhanced black soldier fly larvae meals in broiler chicken: current evidence and future perspectives

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Insect meal is a promising alternative protein for broiler chickens that may also provide functional benefits through bioactive compounds. Research shows that the insects' rearing substrate significantly influences their nutritional and functional quality. Growing insects on agro-industrial by-products, particularly olive oil residues rich in polyphenols can transform low-value waste into high-value feed with enhanced antioxidant, anti-inflammatory, and gut-health properties for poultry. Special attention is given to emerging strategies aimed at improving insect meal functionality through substrate manipulation, including the use of olive oil by-products as explored within the framework of the OLIWA project (https://www.oliwa-project.xyz/). Within the OLIWA framework we will develop *in vivo* broiler trials evaluating graded inclusion levels of functionally enhanced insect meals, alone and in combination with olive waste natural extracts, under commercial-like conditions. Such studies should integrate performance metrics with comprehensive assessments of gut microbiota, immune status, oxidative balance and product quality. These efforts will be essential to validate the practical applicability of nutraceutical-enriched insect meals and to support their adoption as cornerstone ingredients in sustainable broiler nutrition. Additionally, economic and environmental assessments should accompany biological evaluations to quantify sustainability gains, circularity benefits and cost-effectiveness across production chains. Integrating life cycle analysis with nutritional outcomes will further strengthen the case for large-scale implementation of these innovative feed solutions. Acknowledgement: This research is supported by the EU-PRIMA program — project OLIWA- Repurposing OLIve WAste in circular economy solutions for feeds, additives, packaging and biogas — GA 2432, Call 2024 Section 1 Food Value Chain in Nexus.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Effect of house cricket or black soldier fly meals dietary inclusion on meat trait of broilers

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Exploring novel feedstuffs in animal nutrition raises the need to understand, not only the effects on animal performance, but how they influence meat quality and technological traits. This study assessed meat quality and physicochemical traits of broilers fed diets containing two different insect meals as alternative protein sources. A total of 108 male broilers reared for a 35-day production cycle, were allocated to three experimental groups (n = 12): a control diet based on conventional protein source (C), a diet containing 10% *Acheta domesticus* meal (AD), and a diet with 10% *Hermetia illucens* defatted meal (HI). Birds were fed the experimental diets *ad libitum* throughout the entire rearing period. At slaughter, the *pectoralis major* muscle was collected for meat traits assessment. The initial and 24-hour pH, colour (L^*, a^*, b^*), water-holding capacity and texture profile were determined. Data were analysed using a Generalized Linear Model in SAS software, considering diet as the main fixed effect. No significant differences were observed across treatments for initial pH ($p > 0.05$). However, the 24-hour pH was significantly higher in meat from broilers fed the HI and AD diets, compared to C (5.84 and 5.83 vs 5.61; $p < 0.001$). Water-holding capacity was improved by insect meal, with lower drip, thawing and cooking losses observed in both AD and HI groups ($p < 0.01$). Concerning texture, hardness was the highest on AD ($p = 0.006$) while no differences were detected for springiness, cohesiveness or chewiness. Both AD and HI diets produced meat with reduced lightness (L^*), indicating a darker breast muscle compared to C ($p < 0.001$). Overall, the inclusion of 10% insect meal modified several technological traits of broiler meat, particularly water-holding capacity and colour, without markedly impairing texture. Results highlight the importance of considering meat quality attributes when introducing novel feedstuffs in animal nutrition and support the potential of insect meals as sustainable ingredients for broiler diets.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Fatty acid profiles of *Hermetia illucens* and conventional fats and their impact on broiler meat quality

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This study investigated the fatty acid profile of *Hermetia illucens* larvae (HI) fat compared with that of selected conventional fats and its subsequent effect on broiler meat quality. A total of 800 one-day-old Ross 308 male chicks were randomly assigned to eight dietary treatments (isoenergetic and isonitrogenous), each featuring a different fat source: HI fat, palm kernel fatty acid distillate (PKFD), soybean oil (SO), poultry fat (PF), palm oil (PO), rapeseed oil (RO), beef tallow (BT) and pig lard (PL). The samples were collected at the end of the experiment, i.e., on the 35th day. Fatty acid analysis revealed that HI fat was notably enriched in saturated fatty acids (SFAs), especially lauric acid (C12:0), followed by myristic and palmitic acids. Among the plant oils, SO was rich in polyunsaturated fatty acids (PUFAs), RO and PKFD were relatively high in monounsaturated fatty acids (MUFAs), and PO contained more SFAs than unsaturated fatty acids (UFAs). In terms of animal fats, PF and PL contained more UFAs than SFAs did, whereas BT had a slightly greater proportion of SFAs than did the other compounds. Meat quality assessment revealed that HI did not significantly influence postmortem pH or the sensory quality of breast meat. Notably, compared with those from the SO group, the breast muscle from the HI group presented significant increases in α-linolenic acid (C18:3 n-3) and total n-3 fatty acids. HI fat also resulted in favourable PUFA/SFA and n-6/n-3 ratios, comparable to those of plant oils and superior to those of PKFD, which elevated thrombogenic and n-6/n-3 indices. Although HI fat shares a high SFA content with fats such as PO and BT, its unique profile, characterised by elevated C12:0, distinguishes it. The findings demonstrate that HI fat can beneficially modify the fatty acid composition of meat while preserving its technological and sensory properties, aligning its meat quality outcomes more closely with those of plant oils than with those of conventional animal fats. Acknowledgement: This work was supported by an OPUS-20 grant titled “The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens” (no. 2020/39/B/NZ9/00237), which was financed by the National Science Center (Poland).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Immunophysiological and digestibility responses of broilers to black soldier fly larvae fat and common dietary fats

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This study evaluated the immunological and physiological effects and nutrient digestibility of feeding broiler chickens a diet containing fat from black soldier fly larvae (*Hermetia illucens*; BSFL) fat in comparison with conventional plant oils and animal fats. In a 35-day experiment, 800 one-day-old male Ross 308 broilers were randomly distributed into eight dietary groups, each receiving one of the following fat sources: BSFL fat (reference group), palm kernel fatty acid distillate (PKFD), soybean oil (SO), poultry fat (PF), palm oil (PO), rapeseed oil (RO), beef tallow (BT) or pig lard (PL). Nutritional assessment revealed that diets containing PL and BT had significantly lower apparent metabolizable energy corrected for nitrogen balance (AMEN) values than did the BSFL group on the 14th and 28th days. By day 35, no significant differences were observed in the nutrient digestibility coefficients. Analysis of the serum revealed that birds fed BSFL fat had elevated concentrations of immunoglobulin A (IgA) relative to those in the SO, PO, and PL groups; higher immunoglobulin G (IgG) levels than those in the PO group; and increased immunoglobulin Y (IgY) and interleukin-6 (IL-6) levels compared with those in the PL group. Furthermore, serum free triiodothyronine (FT3) levels were significantly higher in the BSFL group than in all the other treatment groups. Liver histomorphology and key serum biochemistry markers remained significantly unaffected across groups relative to BSFL. These results indicate that the dietary inclusion of BSFL fat can enhance specific immune and endocrine parameters in broilers compared with several conventional fat sources, suggesting functional advantages that extend beyond basic nutrition. The observed nutrient digestibility and immunophysiological improvements support the potential of BSFL fat as a bioactive and sustainable lipid ingredient in poultry feed. Acknowledgement: This work was supported by an OPUS-20 grant titled “The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens” (no. 2020/39/B/NZ9/00237), which was financed by the National Science Center (Poland).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Growth performance and digestive tract morphology of broilers fed diets with house cricket or black soldier fly meals

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The search for sustainable alternative protein sources for poultry nutrition has intensified due to environmental and economic constraints associated with conventional soybean meal-based diets. Insect meals have emerged as promising ingredients because of their nutritional value and low ecological footprint. This study assessed the effects of *Acheta domesticus* (house cricket) and *Hermetia illucens* (black soldier fly) meals on growth performance and morphology of the digestive tract of broilers. One hundred and eight one-day-old male Ross 308 chicks were randomly allocated to three dietary treatments and housed in cages with three birds per cage (12 replicates per treatment). Three diets were formulated: a conventional corn–soybean meal diet (C), a diet with 10% *A. domesticus* meal (AD) and a diet with 10% defatted *H. illucens* larvae meal (HI). Diets were offered *ad libitum* over a 35-day experimental period. Body weight and feed intake were recorded weekly and the average daily gain and feed conversion ratio (FCR) were assessed. At 35 days of age, birds were slaughtered, carcasses were dissected and internal organs removed, emptied and weighed. The length of the intestinal compartments and the small intestine digesta viscosity were determined. Carcass yield was assessed by weighing the eviscerated carcass and the main commercial cuts. Data were analysed using the GLM procedure in SAS statistical software. The FCR did not differ between C and HI (1.54 and 1.52), whereas a lower FCR was observed in AD (1.43, $p < 0.001$). In both insect meal-fed groups, carcass and breast yields were higher, glandular stomach and intestinal sections were lighter and shorter, and heart and pancreas weights were lower ($p < 0.001$). Digesta viscosity was not affected ($p > 0.05$). In conclusion, the inclusion of 10% *A. domesticus* or *H. illucens* meal improved growth performance and carcass yield and induced digestive tract adaptations without negative effects on digesta viscosity, supporting the use of both insect meals as sustainable protein sources in broiler nutrition.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Dietary inclusion of up to 20% house cricket meal on broilers growth diet: Impacts on growth performance

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The increased demand for animal-based products has generated pressure on conventional feedstuffs currently used in animal production. It is therefore essential to establish alternative protein sources for monogastric feeding. Insects have gained interest in poultry nutrition as a substitute for soybean meal, due to their nutritional composition with high protein, unsaturated fatty acids profile, as well as its local production. This study aimed to determine the inclusion level of *Acheta domesticus* meal in broiler diets that best favours their growth performance, replacing soybean meal. Three isoproteic and isoenergetic growth diets were formulated based on the nutritional needs of the strain and prepared with 0% (CM0, control), 10% (CM10) and 20% (CM20) cricket meal dietary inclusion. Seventy-two one-day-old, fast-growing male broilers (Ross 308) were allocated to 24 pens, each with three chicks. During the first two weeks, all broilers were fed ad libitum with a control starter diet. Then, each pen was randomly assigned to one of three experimental growth diets (n = 8). The experimental diets were provided ad libitum for three weeks. Liveweight (LW) and feed intake (FI) were recorded weekly and used to calculate average daily gain (ADG) and feed conversion ratio (FCR). Statistical analysis was performed using Analysis of Variance (ANOVA) model in the SAS statistical software, with the diet as the fixed effect and the pen as the experimental unit. Broilers fed diet CM20 showed greater final LW (1429.6 g) and ADG (52.9 g/day) compared with CM10 and control groups (1314.9 and 1352.6 g; 47.7 and 49.7 g/day, respectively). No significant differences were observed on FI and FCR among experimental diets ($p > 0.05$). The results of this trial suggest that the incorporation level of up to 20% of *A. domesticus* meal into broilers diet from day 14 to 35, as a partial substitute for soybean meal, does not have negative impacts on growth performance. Indeed, inclusion of 20% cricket meal in the growth diet improved LW and ADG. However, further studies are needed to fully assess the effects of such incorporation level on carcass yield and meat quality traits.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Replacing common dietary fats with insect fat: effects on broiler growth performance and the gut microbiota

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This study evaluated the effects of replacing selected conventional dietary fats with black soldier fly larvae (BSFL) fat on the growth performance and caecal microbiota of broiler chickens. A total of 800 one-day-old Ross 308 chicks were randomly allotted to eight dietary treatments, each containing a different fat source: soybean oil (SO), rapeseed oil (RO), palm oil (PO), palm kernel fatty acid distillate (PKFD), poultry fat (PF), pig lard (PL), beef tallow (BT), and BSFL fat, which was used as a reference group. The birds were fed ad libitum over a 35-day trial. The results showed that BSFL fat inclusion did not significantly affect body weight gain or feed intake. However, the BSFL group presented a significantly improved feed conversion ratio than the PO, PF and BT groups did. No significant differences were observed in organ weights, immune organ indices, or most gastrointestinal tract segment lengths, except for a lower jejunal weight in the PKFD group than in the BSFL group. Analysis of the caecal microbiota revealed no significant shifts in key bacterial groups between BSFL fat and other dietary treatments. The total microbial counts were greater in the PF and PL groups than in the BSFL group. In conclusion, BSFL fat supported comparable growth performance and gut microbial stability, performing more similarly to plant oils than to animal fats, and thus represents a viable and sustainable alternative lipid source in broiler nutrition. Acknowledgement: This work was supported by an OPUS-20 grant titled “The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens” (no. 2020/39/B/NZ9/00237), which was financed by the National Science Center (Poland).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Caecal microbiota responses to insect-based and conventional fat sources in broilers

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The effects of dietary *Hermetia illucens* larvae (HI) fat on the gut pH and caecal microbiota of broiler chickens were investigated via next-generation sequencing and compared with those of standard plant and animal fats. Ross 308 broilers (n = 800, 10 replications with 10 birds in each replication) were fed nonisoenergetic diets containing one of eight lipid sources as the sole added fat for 35 days: HI fat, soybean oil (SO), rapeseed oil (RO), palm oil (PO), palm kernel fatty acid distillate (PKFD), poultry fat (PF), pig lard (PL) or beef tallow (BT). At the end of the trial, the digesta from the crop, gizzard, jejunum, and caeca were collected for analysis. Compared with the PKFD, PF and BT diets, the HI fat diet significantly lowered the crop pH and increased the gizzard pH. Analysis of the caecal microbial community via 16S rRNA gene sequencing revealed that the alpha diversity metrics did not differ among the treatments, suggesting similar microbial richness and evenness. Beta diversity analysis indicated a significant compositional difference solely between the BSFL and PKFD groups, with no clear differences observed between BSFL fat and the other dietary fat sources. Notably, compared with the PKFD diet, the BSFL diet reduced the relative abundances of Proteobacteria, Enterobacteriaceae and *Escherichia–Shigella*. Linear Discriminant Analysis Effect Size (LEfSe) analysis further revealed that BSFL fat promoted microbial taxa associated with beneficial outcomes, such as Rikenellaceae, Peptococcaceae, *Alistipes*, and the *Eubacterium coprostanoligenes* group, all of which are linked to short-chain fatty acid production and gut health. In summary, HI fat modulated the gastrointestinal pH and promoted a cecal microbiota profile comparable to that of conventional fats while selectively suppressing potential pathogens compared with PKFD. These results support HI fat as a functional and sustainable alternative lipid in broiler nutrition. Acknowledgement: This work was supported by an OPUS-20 grant titled “The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens” (no. 2020/39/B/NZ9/00237), which was financed by the National Science Center (Poland).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Black soldier fly larvae meal as a partial soybean meal replacement in broiler diets: meat quality and sensory traits

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Insects have emerged as a sustainable alternative protein source for animal feed, offering the potential to reduce the environmental footprint of livestock production. This study investigated the effects of partially substituting soybean meal protein with black soldier fly larvae (*Hermetia illucens* L.; BSFL) meal in broiler diets. It assessed impacts on growth performance, carcass and organ traits, meat physicochemical properties, and sensory quality. A total of 120 broiler chickens were randomly assigned to three dietary treatments in a 42-day feeding trial: a control diet (BSFL0), and diets in which BSFL meal replaced 15% (BSFL15) or 65% (BSFL65) of the soybean meal protein. Growth parameters were recorded weekly. Post-slaughter analyses included carcass yield, relative weights of selected organs, and evaluation of breast meat for pH, colour, cooking loss, and basic chemical composition (moisture, protein, fat). Sensory evaluation was conducted on sous-vide-cooked breast meat using a quantitative descriptive analysis by a trained expert panel (n = 9) and consumer hedonic test (n = 29). Results indicated that BSFL inclusion linearly reduced final body weight ($p < 0.001$) and feed intake ($p = 0.011$), with the BSFL65 group exhibiting a significantly higher feed conversion ratio ($p = 0.012$). Behavioural observations suggested altered feeding patterns, particularly at 65% replacement, including increased drinking and selective feed intake, partly associated with a less cohesive feed texture, which likely contributed to reduced feed intake and lower final body weight. Organ weights were affected, with lower relative gizzard and spleen weights in BSFL-fed groups ($p < 0.05$), and signs of early hepatic lipidosis in the BSFL65 birds. Meat quality was altered: BSFL65 breast meat had a lower early post-mortem pH ($p = 0.004$) and showed a lighter colour with decreased redness and yellowness ($p < 0.05$). Cooking loss tended to increase with BSFL inclusion. Notably, the proximate composition of the meat and its overall acceptability by consumers were not significantly different among treatments ($p > 0.05$). However, the expert panel detected distinct sensory profiles, describing BSFL15 meat as softer with a more pronounced aroma, and BSFL65 meat as having the most intense aroma. In summary, while high-level BSFL meal inclusion negatively affected broiler performance and modified specific meat quality traits, overall sensory acceptance remained unchanged. A moderate inclusion level (15% replacement) appears to offer a viable compromise, supporting sustainability initiatives without severely compromising production metrics.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Modelling the metabolizable energy of black soldier fly larvae fat in broiler diets

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This study validated a metabolizable energy (ME) regression model for black soldier fly larvae (BSFL) fat in precision feeding for broilers and assessed its effects on physiological responses. A 28-day trial was conducted with 300 seven-day-old male Ross 308 broilers randomly assigned to two isoenergetic and isonitrogenous dietary treatments: a control diet with soybean oil (SO) or a diet in which SO was fully replaced with BSFL fat as the sole lipid source. The ME values for BSFL fat were applied dynamically per feeding period via a previously established regression model. The results revealed no significant differences in final body weight, feed intake, or feed conversion ratio between the BSFL and SO groups. However, BSFL treatment significantly increased the coefficients of apparent ileal digestibility (CAID) for crude protein and apparent ileal digestible energy (AIDE). Pancreatic lipase activity was also elevated in the BSFL group. Serum analysis revealed that BSFL fat significantly reduced immunoglobulin G (IgG) and thyroxine (T4) concentrations and tended to decrease aspartate aminotransferase (AST), free T4 and interleukin-6 (IL-6) levels, suggesting potential benefits for liver function and immune modulation. Organ morphometry and gastrointestinal pH remained largely unaffected. The study concludes that the applied ME regression model for BSFL fat is effective, supporting comparable growth performance while enhancing protein and energy digestibility.
Acknowledgement: This work was supported by an OPUS-20 grant titled “The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens” (no. 2020/39/B/NZ9/00237), which was financed by the National Science Center (Poland).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Beldi chickens meets *Hermetia illucens*: growth performance, welfare and meat quality

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Poultry meat is a key source of high-quality protein; however, the sector faces challenges in developing affordable and nutritionally adequate alternative feeds, particularly in lower-middle-income countries such as Morocco. Hence, insect-enriched diets with black soldier fly (*Hermetia illucens*, HI) have emerged as a promising solution. This study, conducted for the first time in Morocco, aimed to assess the impact of live HI larvae inclusion on the Beldi chicken performance, welfare and meat quality. A total of 72 four-weeks-old male birds were assigned for 50 days to 3 diets (3 replicate pens/diet and 8 birds/pen): C (control, with 90% of local core mash feed and 10% of feed mix mimicking the larval nutritional profile), HI5 and HI10 (containing 5 and 10% of live HI larvae calculated in dry matter (DM) on the daily feed intake of the birds as partial or complete replacement of the feed mix, respectively). Growth performance (pen level), and plumage status, slaughtering performance and breast physical quality (4 birds/replicate), were evaluated. Data were analysed using IBM SPSS software (v.26, $P < 0.05$). Footpad dermatitis and plumage condition remained optimal and were not affected by diets ($P > 0.05$), with no mortality being recorded. Growth performance did not differ among diets, although birds in the HI5 group exhibited a numerically higher final body weight than the other diets ($P > 0.05$). Slaughtering traits were not influenced by diets, even if cold carcass weight and liver relative weight showed numerical increases in the HI5 and C groups, respectively ($P > 0.05$). No significant differences were observed in post-mortem or ultimate pH values, although increased numerical trends were detected in the HI5 and HI10 groups at 20 min post-mortem and after 24 h at 4 °C ($P = 0.081$ and $P = 0.097$, respectively). From these results, it can be assumed that including HI larva at 5 or 10% did not adversely affect growth performance, welfare status, or carcass traits of Beldi birds and it can be considered a suitable diet ingredient. Further studies should assess HI effects on meat quality, sensory properties and its use at early growth stages at different inclusion levels. Acknowledgement: This research is being funded in Morocco by MENFPESRS/DESRS and supported by PRIMA (ADVAGROMED project).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Live *Tenebrio molitor* larvae as feed ingredients for native chickens: gut health effects

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Live insect larvae constitute a valuable environmental enrichment for native chickens, but their use as feed ingredient has not been investigated. This study aimed to assess the effects of live *Tenebrio molitor* (TM) larvae on gut health of Bionda Piemontese native hens. 40-week-old birds (n = 198) were allotted to 3 diets: C (control), TM5 and TM10 (containing 5% and 10% of live TM larvae calculated in dry matter on daily feed intake, respectively). After 12 weeks, 12 hens/diet were slaughtered, and gut segments, liver and spleen were collected for histomorphometry and histopathology. The evaluated morphometric indices were villus height (Vh), villus width (Vw), crypt depth (Cd), Vh/Cd, mucosal (MT), submucosal (SmT) and muscularis (MuT) thickness. Jejunum sections were also stained with periodic-acid Schiff, Alcian Blue pH 2.5 and High-Iron Diamine to characterise mucins. A semiquantitative scoring system was used to assess inflammation severity (gut segments, liver, spleen), pattern and type (gut segments), degeneration (liver), lymphoid hyperplasia and depletion (spleen), and intensity of histochemical stains in crypts and villi. Quantitative real-time PCR (qPCR) was performed on jejunum to assess the gene expression of pro- (IL-2, TNF-α, INF-γ) and anti-inflammatory (IL-4) cytokines, mucins (MUC-2), and tight-junctions (ZO-1, CL-1). No significant differences were observed between dietary groups for any morphometric, histopathological and histochemical parameter analysed ($p > 0.05$). Conversely, the relative mRNA expression of IL-4 was significantly higher in TM groups when compared to C ($p = 0.039$), while that of all other evaluated genes was not influenced by the diet ($p > 0.05$). Overall, the use of live TM larvae as feed ingredient for native chickens showed no negative impact on the morphopathological parameters analysed herein and may promote an anti-inflammatory intestinal status. Correlation of such results with the animals' productive performance, egg quality, gastrointestinal and hepatic enzymatic activity, and microbiota is ongoing. Acknowledgements: The research is supported by the EU-PRIMA program project ADVAGROMED (PRIMA, 2021-section 2). The project is funded by the Ministry of Universities and Research (MUR) under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union's Programme for Research and Innovation.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Feeding live black soldier fly larvae to free-range Lohmann Brown laying hens: effects on performance and egg quality

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Egg production faces increasing demands to improve sustainability, especially by reducing reliance on imported protein sources like soybean meal. This study investigated the effects of feeding live black soldier fly larvae (LBSFL) as a substitute for soybean meal (SBM) and soybean oil (SO) on the laying performance, egg quality traits, and fatty acid composition of the egg yolk of Lohmann Brown laying hens. A total of 144 18-week-old hens were allocated equally (4 pens/treatment, 18 hens/pen, free-range system) to two dietary treatments: SOY and LAR. Birds in the SOY group received a commercial laying diet with 15% SBM+SO during phase 1 (P1, 18 – 39 weeks) and 10% SBM+SO in phase 2 (P2, 40 – 50 weeks), while SBM+SO was completely replaced by LBSFL in LAR treatment. LBSFL was offered twice a day based on expected dry matter intake. Growth performance and feed intake were recorded weekly, laying performance daily, and egg quality weekly (P1) or every two weeks (P2). Yolk fatty acid composition was analyzed at the end of each phase. Data were analysed using one-way ANOVA ($p < 0.05$). Feeding LBSFL did not affect hens' growth and laying performance ($p > 0.05$). During the whole trial period, BSFL-fed hens showed a significantly reduced overall dry matter intake ($p < 0.05$) and a higher average individual egg weight ($p < 0.05$), resulting in a tendency toward improved feed conversion ratio ($p < 0.1$). During the entire trial period, there were no significant differences in the egg quality parameters such as shape index, Haugh units, amount of yolk, pH value in yolk and albumen, and yolk index between both treatments (n = 2296). However, individual egg weight and yolk colour ($p < 0.05$) increased significantly, while shell thickness ($p < 0.05$) and amount of shell weight ($p < 0.05$) were reduced in LAR. LBSFL feeding increased the saturated fatty acid (SFA) content and reduced the polyunsaturated fatty acid (PUFA) proportion in LAR-yolk, compared to SOY, while total FA and monounsaturated FA (MUFA) were unaffected. The increase in SFA was primarily due to higher levels of C12, C14 and C16:0, which are the main FAs present in BSFL. The results of this study indicate that replacing SBM+SO with live BSFL is a suitable and sustainable alternative protein and energy source for laying hens, while maintaining production performance and modifying egg yolk FA composition.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Black soldier fly larvae in slow-growing chicken diets: effects on growth, gut health and digestive enzyme modulation

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Black soldier fly larvae (BSFL) supplementation in chicken diets has been proposed as a sustainable protein source rich in nutrients. This study evaluates the impact of supplementing dehydrated and live BSFL reared on Gainesville substrate (D-BSFL and L-BSFL, respectively) to a slow-growing chicken breed called Bianca di Saluzzo (BS). A total of 144 BS males (39 days old) were distributed in 18 pens (3 treatments/6 replicates). The groups were categorized as: (1) Control Group (C) fed a basal diet (0% soybean meal substituted with alternative protein ingredients); (2) DL group fed the C diet supplemented with 5% D-BSFL; (3) LL group fed the C diet supplemented with 5% L-BSFL. The larvae consumption time was recorded weekly. Feed intake was recorded weekly and larvae were provided at 5% of dry matter intake. Birds were weighed and feed conversion ratio calculated every 21 days. At 147 and 174 days, two birds per replicate (72 total) were slaughtered. Five-cm segments of the duodenum, jejunum, ileum, spleen and left liver lobe were collected for histological and histopathological evaluation. Colloidal chitin was incubated with proventriculus enzyme extracts to assess endo-, exo-chitinase, and chitosanase activities. Levene's test was used to assess variance homogeneity, considering pens for growth performance and individual birds for gut analyses. Data were analyzed using a Generalized Linear Model (RStudio). DL and LL groups showed higher feed intake and 4% higher final live weights compared to C (C: 2335 g; DL: 2440 g; LL: 2412 g). However, no differences were noted in FCR among the groups. The supplementation of BSFL did not highlight alterations in intestinal morphometry or histopathological severity in any of the sampled organs confirming the safety of BSFL supplementation for chickens. Additionally, animals fed BSFL showed markedly higher endo-chitinase and exo-chitinase activities than controls (+96.9% and +48.2%, respectively), whereas chitosanase activity remained unchanged, indicating that BSFL inclusion selectively modulates chitinolytic enzyme activity. In conclusion, the birds demonstrated good adaptability in response to the supplementations of BSFL, with no differences at gut level between live or dehydrated larvae. Overall, such adaptive responses underscore the capacity of slow-growing breeds to adjust and optimize their dietary strategies in response to alternative feeding regimens, appearing particularly suitable for extensive and slow-growing production systems, where birds' adaptive capacity and longer rearing periods allow full nutritional and physiological benefits to emerge.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Impact of live insect larvae on welfare indicators and meat traits in Muscovy ducks

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The aim of the present study was to evaluate the effects of dietary supplementation of live black soldier fly (BSF) and yellow mealworm (YMW) larvae on welfare, slaughter performance, meat quality, proximate composition, and fatty acid profile of Muscovy ducks. A total of 126 female Muscovy ducks (average live weight, LW: 80.3 ± 7.28 g) were divided into three experimental groups: control (commercial feed), BSF (commercial feed + 5% BSF larvae), and YMW (commercial feed + 5% YMW larvae). A 2-phase feeding program was applied (3–31 and 32–55 days of age for starter and grower-finisher, respectively). During the trial, larva consumption times were recorded daily. Excreta corticosterone metabolites (ECM) were assessed at 3, 31 and 55 days of age. At 55 days of age, the final LW was recorded then 12 birds/treatment were selected and blood samples were collected. Selected birds were slaughtered and meat quality traits were evaluated. The experimental treatments did not affect growth performance of the birds ($P > 0.05$). Larva consumption time was generally similar between the two insect species, except between 14 and 18 days of age, when BSF larvae were consumed faster than YMW larvae ($P < 0.001$). The provision of live BSF and YMW larvae significantly reduced the ECM at 55 days of age and the heterophil to lymphocyte ratio ($P < 0.05$). No significant differences were observed among groups regarding the slaughter performance or overall meat quality traits, with the exception of thigh meat proximate composition. Specifically, thigh meat from insect-fed ducks showed a lower moisture content and a higher crude protein content compared with the control group ($P < 0.05$). Regarding the fatty acid profile, meat from BSF-fed ducks exhibited a higher proportion of C12:0. Moreover, both insect-fed groups showed higher C14:0 and C16:1 n-7 amount in the thigh meat compared with control ($P < 0.05$). Overall, the obtained findings confirm that the dietary supplementation of live BSF and YMW larvae in Muscovy duck does not compromise the meat quality and may positively influence welfare-related indicators.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Impact of full-fat insect meal on egg production, quality, hatchability, and physicochemical traits of pheasants

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This study examined the effects of full-fat *Hermetia illucens* (BSFL) larval meal in pheasant diets on egg production, hatchability, and physicochemical traits. BSFL provides sustainable protein to increase poultry performance. Seventy-two pheasants (63 females, 9 males) were randomised into three groups: BSFL0 (control), BSFL10 (basal + 100 g/kg BSFL), or BSFL20 (basal + 200 g/kg BSFL). The production indices of 1200 viable eggs were analysed. The data were compared via one-way ANOVA, with significance at $P < 0.05$. The BSFL20 group yielded the most eggs (469; $P = 0.001$), the fewest rejections (3.7%; $P = 0.041$), the highest albumen height (4.36 mm; $P = 0.038$), and the highest Haugh index (76.3; $P = 0.046$). Chick survival showed positive trends up to day 7 ($P = 0.068$) and day 14 ($P = 0.061$) with higher BSFL doses. BSFL20 yolks presented relatively high dry matter and protein contents — 464 g/kg and 158 g/kg egg yolk, respectively ($P < 0.05$) — and tended toward relatively low cholesterol levels ($P = 0.067$). With respect to the fatty acid profiles of the experimental groups, the C12:0 and C14:0 ($P < 0.05$) levels increased; however, the C16:1 ($P = 0.021$) and monounsaturated fatty acid levels ($P = 0.024$) decreased. Additionally, BSFL20 presented a reduced C18:0 level of 8.07% ($P = 0.042$). The atherogenic index increased to 0.56 ($P = 0.038$), whereas the n-6/n-3 ratio decreased to 7.83 ($P = 0.037$) in BSFL20 versus the control. Pheasants naturally consume insects with grains, yet modern diets lack these protein-rich sources, limiting reproduction amid agricultural shifts. The inclusion of full-fat *H. illucens* meal at 200 g/kg increased egg number and quality (Haugh index, albumen height) and promoted positive chick survival trends without affecting body weight or hatchability due to the balance of protein and energy. It also enriched yolks with dry matter, protein and favourable fatty acids (elevated C12:0/C14:0, reduced n-6/n-3 ratio), positioning BSFL as a sustainable 200 g/kg additive for pheasant production.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Enriched farming systems and *Tenebrio molitor* supplementation of pheasant (*Phasianus colchicus*) raised for restocking

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The common pheasant (*Phasianus colchicus*), native to regions from the Caucasus to Eastern China, is now a major game bird in Europe and North America. Most countries rely on captive-reared birds for stocking to sustain hunting and maintain populations in managed habitats. The present study aimed to evaluate the effect of supplementing live *Tenebrio molitor* (TM) larvae and enhanced rearing protocols in pheasant chicks reared for restocking. 40 pheasant were weighed and tagged individually and divided in a Control Group (C, 10 males and 10 females) fed with standard crumbled feed, raised without environmental enrichment and an Experimental group (EXP, 10 males and 10 females) fed with supplementation of 1,6% of live weight of TM larvae administered daily, *ad libitum* bran availability, high perches and raised in a grass-covered aviary. Every 14 days, live weight (LW) was recorded. At 91 days, the birds were slaughtered. Gut, organs and carcass weight (CW) were recorded. A GLM was done to assess the effects of Treatment, Sex, and their interactions. Sexual dimorphism emerged as a key factor shaping growth trajectories, with significant differences between males and females appearing at day 35 (p = 0.0137) and progressively increasing over time. Males reached 35% higher LW than females. This dimorphism was also reflected in CW (+33%), whereas relative proportions of breast, thigh, and bone were consistent between sexes. Treatment effects were sex-dependent, as indicated by significant Treatment × Sex interactions from day 49 onward. Males responded more strongly to experimental treatments, achieving 1042.4 g compared to 935.2 g in controls (+11.5%), while females exhibited only a minimal response (742.0 g vs 722.6 g, +2.7%). Survival analysis indicated that enhanced rearing improved survival rates (85.7% vs 57.9%), although this difference did not reach statistical significance (p = 0.107), likely due to the limited sample size. These results indicate that rearing responses in common pheasant are markedly sex-dependent, with males exhibiting higher growth rates and stronger responses to treatments. Such sex-specific effects are crucial for designing targeted rearing protocols and improving production systems for both conservation and commercial purpose.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Productive and physiological responses of the neotropical fish *Piaractus brachypomus* fed black soldier fly larval meal

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The rapid expansion of aquaculture in South America positions cachama blanca (*Piaractus brachypomus*) as a tropical species with strong productive potential for domestic consumption and export markets. However, in Colombia, feed cost represent 50 to 70% of total production expenses, largely due to dependence on imported ingredients such as soybean products and fishmeal. The black soldier fly (*Hermetia illucens*), a species native to tropical regions, has therefore emerged as a promising alternative protein and lipid source for more sustainable aquafeeds. This study evaluated the effects of including full-fat *H. illucens* larval meal as a partial substitute for soybean meal + soy oil in isonitrogenous and isoenergetic diets for fingerlings. Four dietary treatments (0, 5, 10 and 15% inclusion) were tested with four replicates each in a recirculating aquaculture system comprising 16 tanks (70 L) stocked with 96 fish (initial weight 5.82 ± 2.84 g) over 55 days. Productive performance was assessed through feed intake (FI), feed conversion ratio (FCR), absolute and specific growth rates (AGR and SGR), and somatic indices, including hepatosomatic (HSI), viscerosomatic (VSI) and visceral fat indices (VFI). Plasma cholesterol and triglycerides, carcass yield, and proximate composition were also determined. No significant differences were observed among treatments for FCR, AGR, SGR, HSI, VSI, VFI, plasma lipids or carcass yield. Carcass nutrient composition was similarly unaffected, with dry matter ranging from 31.10 ± 0.62 to 31.80 ± 0.29%, crude protein from 15.75 ± 0.29 to 16.28 ± 0.15%, ether extract from 10.50 ± 0.41 to 11.15 ± 0.91%, ash from 2.90 ± 0.12 to 2.98 ± 0.17%, calcium from 0.75 ± 0.02 to 0.81 ± 0.03% and magnesium at approximately 0.04%. Gross energy values ranged between 1.95 ± 0.06 and 1.98 ± 0.10 Mcal kg^{-1}. Phosphorus content differed slightly at the 10% inclusion level (0.57 ± 0.01). Feed intake varied among treatments, with the 10% inclusion diet showing higher consumption without compromising growth performance or physiological indicators. These results indicate that full-fat *H. illucens* larval meal can be incorporated at levels up to 15% in diets for *P. brachypomus* fingerlings without negative effects on growth, carcass composition, or health-related parameters, supporting its potential as a sustainable ingredient for tropical aquaculture feeds.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Effect of *Tenebrio molitor* derived protein on growth and immune resilience of European seabass (*Dicentrarchus labrax*)

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The disposal of adult insects will increase rapidly along with continuous expansion of insect industry. Despite the high protein, chitin of insects impairs digestibility, hindering the use in feeds. This work aims to separate and use protein from *T. molitor* adults (beetles) as a functional ingredient in fish diets. Also, part of protein was encapsulated with chitosan and tested as functional ingredient. Proteins were incorporated in diets for European seabass juveniles, carrying out a growth trial followed by exposing fish to thermal stress, assessing potential immune boosting effects associated with the novel ingredients. Beetles protein was extracted using betaine and urea solvent (1:2 mol ratio; 90 °C, 3 h). Encapsulation was carried out using 2.5% (w/w) commercial chitosan in 2% (v/v) acetic acid. Three diets were formulated: control (no beetles' protein), N-ENC (1% DW non-encapsulated protein) and ENC (1% DW encapsulated protein). Fish were fed for 61 days, assessing growth, and then submitted to thermal stress (water temperature increase up to 30 °C, 15 days), evaluating immune-related gene expression. The novel ingredients did not affect fish growth, feed efficiency (varying between 0.77 ± 0.10 in control and 0.83 ± 0.09 in the N-ENC group), and protein efficiency ratio (varying between 1.64 ± 0.22 in ENC group and 1.75 ± 0.20 in N-ENC). No differences were observed in plasma metabolite profile and oxidative stress enzymes. Lower proinflammatory-related genes expression was observed in N-ENC compared to the control and ENC groups. After thermal stress, the N-ENC group exhibited higher activities of oxidative stress-related enzymes in intestine compared to ENC group. The expression of IL-β, Cox-2 and TGF-β genes was higher in control, decreasing in the ENC and N-ENC groups. The expression of TNF-α, CASP-3 and IL-10 genes was higher in N-ENC compared to the ENC group. Beetles' protein did not compromise growth and feed utilisation and induced anti-inflammatory effects in fish submitted to chronic stress compared to the control. Still, the dietary inclusion of encapsulated protein compromised fish immune defense. The dietary inclusion of non-encapsulated beetles' protein positively impacts the immune response of European seabass exposed to chronic stress.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Valorisation of by-products via *Tenebrio molitor* bioconversion for sustainable aquafeeds development

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SAFE (Smart Aqua for Future) is an EU-funded Horizon Europe project promoting circular economy models to improve the sustainability of freshwater aquaculture in Europe. A core objective is the valorisation of solid and liquid waste streams to reduce environmental impact and support sustainable aquafeed production. In this context, insects, particularly the yellow mealworm (*Tenebrio molitor*), offer an efficient pathway to convert low-value side streams into high-quality feed ingredients. Duckweed (*Lemna* spp.), watercress (*Nasturtium officinale*) and Spent Mushroom Substrate (SMS) mixed with aquaculture residues were tested as rearing substrates for *T. molitor* larvae at increasing inclusion levels. Larval growth and composition were analysed. The best-performing substrate was upscaled, and the resulting mealworm meal was evaluated in two feeding trials with juvenile rainbow trout (*Oncorhynchus mykiss*), assessing incremental fishmeal replacement with mealworm meal reared on different substrates, and total replacement with either full-fat or defatted mealworm meals. Growth, nutrient digestibility, digestive enzyme activity, nutrient retention and fillet composition were measured. Inclusion of aquatic plants in mealworm substrate reduced larval intake and growth, whereas SMS-based diets increased larval biomass up to 14% over control diets. In rainbow trout, mealworm meals supported fish growth comparable to fishmeal. Total fishmeal replacement reduced feed intake while significantly improving feed and protein efficiency ratios. Mealworm meals inclusion increased apparent digestibility of dry matter, protein, ash, phosphorus and energy, together with fish intestinal trypsin activity. Fillet composition was largely unaffected, but defatted mealworm meal improved lipid quality compared to full-fat treatment. Bioconversion of aquaculture and agro-industrial residues by *T. molitor* enables the production of high-quality insect meals for aquafeeds. Substrate selection and processing strategy importantly influence nutritional and functional outcomes. This approach supports nutrient recycling and the use of mealworm meal as part of sustainable circular aquaculture systems. This research was part of SAFE Project.

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Metagenomic insights into gut microbiota modulation by black soldier fly larvae in three Australian aquaculture species

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The fish gut microbiota is integral to nutrient metabolism and influences key physiological processes including growth, immune function, and disease resistance. Understanding and modulating these microbial communities offers significant potential for improving aquaculture productivity and fish health. This study investigated the effects of dietary inclusion of black soldier fly larvae meal (*Hermetia illucens*, BSFL) on the gut microbiome of three Australian finfish species: *Maccullochella peelii* (Murray cod), *Bidyanus bidyanus* (silver perch), and *Tandanus tandanus* (Australian catfish). Diets were formulated to reflect natural insect prey profiles observed in wild populations and were compared against conventional commercial diets over a 10-week feeding trial. Gut microbial communities were characterised using Illumina deep sequencing of the V1–V2 hypervariable region of the 16S rRNA gene, followed by bioinformatic analysis. A total of 5 110 515 reads were taxonomically assigned using the SILVA database, yielding 1728 operational taxonomic units (OTUs) at 97% similarity across 87 samples. Core gut bacteria across species were dominated by genera including *Plesiomonas*, *Aeromonas* and *Vibrio*. Growth performance improved across all species, with Australian Catfish showing significantly enhanced growth relative to commercial diet controls ($p < 0.05$). Metagenomic analysis revealed that dietary BSFL induced distinct shifts in microbial community composition within each species, differentiating them from baseline microbiota and commercial diet controls. These microbial changes were associated with enhanced nutrient utilisation, supporting the hypothesis that BSFL meal modulates gut microbiota in ways that promote efficient nutrient conversion. Overall, this study demonstrates that BSFL meal is a promising alternative protein source capable of beneficially altering gut microbiota and improving growth performance in multiple Australian freshwater fish species, with implications for sustainable aquaculture nutrition strategies.

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Assessing the digestibility of home-made insect meals as feed ingredients for rainbow trout (*Oncorhynchus mykiss*)

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Digestibility is a key component in assessing the nutritional value of novel aquaculture feed ingredients. However, digestibility of nutrient of feed ingredients may vary depending on raw material origins and processing conditions. This study examined the digestibility of *Hermetia illucens* (HI) and *Tenebrio molitor* (TM) meals fed to rainbow trout (*Oncorhynchus mykiss*). To minimise bias, all the production steps from larval rearing and processing to digestibility trial were conducted within the DISAFA experimental facility (University of Turin, Italy). Fresh larvae (HI 335 kg; TM 306 kg) were reared on agro by-products (HI mainly on brewer's grain, dry distillery stillage and rice by-products; TM mostly on feed waste, breading waste and rice by-products), boiled (1:1 water:larvae approx. 15 min), and dried (70 °C approx. 18 h). Dried larvae were mechanically pressed (meal yield: HI, 68.5%; TM, 79.9%) and chemically analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash and gross energy (GE, MJ/kg). The HI meal (DM 96.7, CP 53.0, EE 14.9, ash 6.4%DM; GE 23.76 MJ/kg) and TM meal (DM 96.1, CP 58.2, EE 11.6, ash 4.7%DM; GE 23.71 MJ/kg) were evaluated as individual ingredients (30% of inclusion) in a digestibility trial using a highly digestible reference diet. A total of 135 rainbow trout (238,0 ± 6,7g) were distributed into nine cylindrical–conical tanks (Choubert system), with three replicates per treatment and fed manually to apparent satiety twice daily (09:00 and 15:00). After a 17-day acclimation period to the tank and diets, faeces were manually collected, freeze-dried and analysed to calculate nutrient apparent digestibility coefficients (ADC). Differences between HI and TM ADC were evaluated using an independent-samples *t*-test (IBM SPSS, $p < 0.05$). No significant differences were observed in the ADC of DM, CP, EE and GE between the two ingredients tested ($p > 0.05$). The HI digestibility values (DM 76.1; CP 91.2; EE 97.1; GE 81.9%) were numerically comparable to those of TM (DM 71.8; CP 92.5; EE 96.1; GE 78.7%). The study provides nutrient digestibility information for HI and TM meals required to formulate diets on a digestible nutrient basis for subsequent growth trials. Acknowledgement: Research supported by Fondazione Cariplo (newRIFF project).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Black soldier fly larvae modulate gut microbiota, inflammation and serum metabolome in yellow catfish

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Bioactive compounds found in black soldier fly larvae (BSFL), including antimicrobial peptides, medium-chain fatty acids, and chitin, may affect intestinal health and host metabolism. We hypothesized that BSFL inclusion can modulate gut microbiota and reduce intestinal inflammation, and thereby induce a shift in serum metabolomic profiles. In a 20-week trial, yellow catfish (32.69 ± 5.90 g initial weight) were fed one of three diets, each with four replicate tanks: a commercial feed (Control; 34.50% crude protein, 4.28% crude fat, 1875.38 kJ/100 g gross energy), or diets where 15% (BSFL15; 35.51% crude protein, 5.98% crude fat, 1902.13 kJ/100 g gross energy) or 30% (BSFL30; 37.47% crude protein, 8.69% crude fat, 1951.73 kJ/100 g gross energy) of it was replaced by partially defatted BSFL meal. Body weight and length were recorded. Serum malondialdehyde (MDA), lipopolysaccharide (LPS), D-lactate, total antioxidant capacity (T-AOC), targeted metabolomics, gut microbiota, and intestinal gene expression of inflammatory cytokines and tight junction proteins were analysed. Final body weights were 67.09 ± 7.13 g (Control), 63.45 ± 4.16 g (BSFL15), and 64.19 ± 3.42 g (BSFL30) ($P > 0.05$). BSFL inclusion did not affect serum markers of intestinal integrity (LPS and D-lactate; $P > 0.05$) but significantly altered oxidative stress markers (increased MDA and decreased T-AOC; $P < 0.05$). Fish fed with 30% BSFL had upregulated expression of pro-inflammatory (TNF-α and IL-6) and anti-inflammatory cytokines (IL-10) in the anterior gut ($P < 0.05$). BSFL inclusion increased gut microbial richness but did not alter the dominant taxa. Serum metabolomics revealed 34 differential metabolites, including medium-chain fatty acids, bile acids, and amino acid-related metabolites. Intestinal microbial genera including *Paraclostridium* and *Epulopiscium* were positively correlated with several differential metabolites, whereas the genus *Romboutsia* was negatively correlated. In conclusion, a 30% BSFL inclusion posed an inflammatory trigger, while 15% inclusion only induced mild oxidative stress. The correlations between serum metabolites and gut microbiota indicate that BSFL influence fish physiology not merely by altering nutrient supply, but also by modulating key bacteria, thereby affecting the host's metabolic pathways related to bile acids, fatty acids and amino acids.

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Insect–algae diets and their impact on rainbow trout growth and fillet quality

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The last decade, there has been a growing need for new aquafeeds ingredients that meet nutritional requirements while reducing environmental impact. Insects and microalgae have emerged as promising alternatives, providing protein and highly unsaturated n-3 fatty acids, respectively. This study evaluated the use of *Hermetia illucens* (HI) and *Spirulina* (SP) meals — individually and in combination — as feed ingredients for *Oncorhynchus mykiss* (rainbow trout), with aim of identify optimal inclusion levels to enhance growth performance and fillet quality. Seven isonitrogenous, isolipidic, and isoenergetic diets were formulated: a control (CTRL) containing 20% of fish meal, and six diets where the fish meal was 100% replaced with *Hermetia illucens* meal (HI100), 100% *Spirulina* meal (SP100), 75% HI and 25% SP (MIX1), 66.6% HI and 33.3% SP (MIX2), 33.3% HI and 66.6% SP (MIX3) and 25% HI and 75% SP (MIX4). A total of 441 fish (initial body weight of 200.5 ± 2.6 g) were randomly allotted to 21 400L-tanks (3 replicates/diet, 21 fish/tank). Feed was hand-distributed twice daily, seven days a week. Daily rations were set as a% of the tank biomass and adjusted every 14 days based on fish growth and water temperature. The trial lasted 88 days. At the end, growth performance indices were calculated, and 10 fish per tank were slaughtered to determine the somatic indices (n = 6) and proximate composition, physical quality, and texture profile of the fillets (n = 4). Using the IBM SPSS Statistics 20 Software, the normality and homogeneity of data were tested using the Shapiro–Wilk and Levene's tests, respectively. In cases where the normality assumption was violated, the Kruskal–Wallis test was used, otherwise one-way ANOVA followed by the Tukey test at $p < 0.05$ was performed. Growth performance was not affected by diet ($p > 0.05$), as well as somatic indices ($p > 0.05$). Regarding the physical quality of the fillets, the pH and water losses were similar for all the diets ($p > 0.05$); however, the redness (a^*) and yellowness (b^*) indices were higher in all the diets containing SP when compared to CTRL and HI ($p < 0.05$), thus being attributable to the SP pigments. The proximate composition, as well as the texture profile analysis, did not show any differences between diets ($p > 0.05$). In conclusion, this study showed that it is possible to replace the fish meal with HI, SP meal, or a combination of these two ingredients without affecting the growth performance and the fillet quality of rainbow trout, apart from the fillet colour in the SP-fed fish. Acknowldgement: Research supported by PRIMA, a program supported by the EU, under GA No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

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Feeding *Colossoma macropomum*, with flour from larvae of *Hermetia illucens*, produced by Peruvian low-income fish farmers

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This project is based on the transfer and implementation of an innovative technique developed by the French Research Institute for Development (IRD) and its Peruvian partners: the Peruvian Amazon Research Institute (IIAP) and Cayetano Heredia Peruvian University (UPCH). It consists of developing a sustainable diet for the cultivation of Gamitana fry, (*Colossoma macropomum*) with Amazonian aquaculture farmers with limited resources, through the cultivation and use of a detritivorous insect native to the Amazon, known as the black soldier fly, *Hermetia illucens*, recognised for its great capacity for food bioconversion of organic waste during its larval stage and for being a protein-rich food source for animal nutrition. The project consisted of four phases: The first phase consisted of selecting 13 pairs of fish farmers to be beneficiaries of the project. For the selection, surveys with certain evaluation criteria were conducted with 145 fish farmers from 15 rural communities in the Peruvian Amazon. The second phase consisted of training the selected fish farmers in the technique of farming and producing MSN larvae meal, preparing diets, and managing the feeding of *C. macropomum* fry using these diets. The third phase consisted of providing each beneficiary's farming centre with the necessary materials to set up a space for farming and producing MSN meal, as well as a floating cage with 300 *C. macropomum* fry to be fed with the prepared diets. At this stage, the application of the techniques taught was evaluated. The fourth phase, was focused on measuring the results obtained in terms of improving the production capacity of each beneficiary. This experience is helping to improve fish production among Amazonian fish farmers with limited economic resources, generating interest among them to organise themselves to carry out this farming process using their own resources, proposing possible alliances among themselves (aquaculture associations) and with the municipalities where they live. We made audiovisual instruments to expend the knowledge to other communities around the World, see https://youtu.be/4fUOS5N7Mbo?si=O6YgLclNm09U0G2S

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Beyond protein: the functional potential of black soldier fly larvae as a sustainable attractant for shrimp aquaculture

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The Pacific white shrimp *Penaeus vannamei* dominates global shrimp aquaculture, driving the need for sustainable and cost-effective feed ingredients. Squid meal has long been used at low inclusion levels as a potent palatant due to its high content of free amino acids, nucleotides, and other water-soluble compounds that stimulate feed detection and intake. However, squid meal and other marine-derived palatants such as krill are subject to high price volatility, variable quality, and increasing ecological and regulatory constraints, prompting the search for alternative attractant sources. Insect ingredients derived from black soldier fly larvae (BSFL, *Hermetia illucens*) represent a promising alternative. BSFL-based ingredients contain a diverse profile of short-chain peptides, free amino acids, and other soluble compounds that may contribute to feed attractiveness. In addition, insect production offers advantages in terms of circularity, supply security, and reduced environmental footprint compared with marine resources. While BSFL meals have been increasingly studied as protein sources in shrimp feeds, their functional potential as palatants remains insufficiently documented. The replacement of squid meal by an industrial BSFL-derived palatant in *P. vannamei* diets was evaluated. Three diets were formulated to meet nutritional requirements while limiting nuisance variables: a negative control diet without palatant, a positive control diet containing 3% squid meal, and a test diet containing 3% BSFL-derived palatant. A total of 300 shrimp were randomly distributed into 12 tanks and fed manually over 17 days. Feed intake was expressed as a percentage of average body weight per day. The BSFL-derived palatant displayed a strong phago-stimulatory effect, significantly increasing relative feed intake by 7.56% compared to the control diet, and demonstrating an attractant effect comparable to squid meal. No significant differences in feed conversion ratio were observed between groups, indicating that both palatant-containing diets maintained feed efficiency while supporting improved growth. Overall, these results support the use of black soldier fly larvae as an effective replacement for traditional marine-derived palatants in shrimp feeds, offering a sustainable and functional solution that supports shrimp performance and the circular economy of aquaculture.

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Comparative effects of black soldier fly and mealworm meal on growth, composition, and antioxidants in striped snakehead

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As aquaculture seeks sustainable protein alternatives to fishmeal, insect-based meals have emerged as promising candidates. This study investigated the efficacy of replacing fishmeal (FM) with black soldier fly larvae (BSFL) and mealworm meal (MWM) in the diet of snakehead (*Channa striata*). A feeding trial was conducted over 8 weeks, during which three experimental diets were formulated: a Control (35% FM), a diet with 10.5% BSFL and 10.5% MWM inclusion, replacing up to 30% of the fishmeal. Fish were evaluated for growth performance, feed utilisation, organosomatic indices and whole-body proximate composition. Additionally, the activities of glutathione s-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) were analysed to monitor antioxidant responses to the insect-based ingredients. Growth performance and feed efficiency varied significantly between the treatments. The BSFL group achieved the highest weight gain (20.17 ± 0.28g) and specific growth rate (SGR) (2.57 ± 0.01% day^{-1}), performing similarly to the control. Conversely, the MWM group showed significantly lower ($p < 0.05$) weight gain and SGR compared to the other diets. The BSFL and control groups maintained a more efficient feed conversion ratio (FCR). Analysis of body composition and antioxidant enzymes revealed that both whole-body protein content and GST activity were significantly higher ($p < 0.05$) in the BSFL group compared to the MWM group. No significant differences were observed in survival rates, organosomatic indices, CAT, or SOD activities across the experimental diets. The findings demonstrate that BSFL is an effective and sustainable substitute for fishmeal at a 30% replacement level, improving growth performance (final weight, weight gain, FCR and SGR), whole-body protein content, and physiological health via enhanced GST activity when compared to mealworm-based diets. Consequently, BSFL meal is a superior functional protein source for improving production efficiency and oxidative status in snakehead culture.

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Functional protein from silkworm enables fishmeal reduction without compromising Atlantic salmon performance

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Silkworm pupae meal, a long-standing co-product of sericulture, is increasingly recognized as a valuable insect-derived protein source for aquafeeds. Its favourable amino acid composition, lipid profile, and generally stable nutrient characteristics make it suitable for reducing dependence on traditional marine proteins. However, protein digestibility remains lower. In this study, a process to enhance the protein and digestibility of silkworm pupae was developed and evaluated for its effectiveness for inclusion in aquafeed as a source of protein replacing animal protein sources such as fish meal. Being derived from a waste by-product, this provides for a sustainable source of protein for aquaculture reducing FIFO ratios. A controlled 4-week feeding study was conducted with juvenile Atlantic salmon (*Salmo salar*) reared in standardized freshwater systems. Diets were produced using a twin-screw extruder with pre-conditioning: a control diet containing 20% fishmeal, and a test diet in which fishmeal was reduced to 15% by incorporating 5% silkworm protein, equivalent to a 25% replacement within the fishmeal fraction. Fish were monitored daily to ensure stable environmental and health conditions throughout the study. Process development has substantially improved the quality of silkworm protein, enhancing the protein content by 7.8%, digestibility by 10.6%, and reducing the fibre content by 0.7%. Across the 28-day feeding period, salmon offered the silkworm protein diet showed growth performance equivalent to the control group. Final body weight, specific growth rate and eFCR did not differ significantly between the groups ($p > 0.05$). The proximate composition of the silkworm protein diet remained well balanced, with crude protein around 53.3% and lipid near 18.8%. These results demonstrate that silkworm protein enables a meaningful reduction in fish meal usage without compromising growth. The findings also emphasize the potential of silkworm protein within circular and resource-efficient feed strategies by converting sericulture byproducts into nutritionally relevant aquafeed ingredients. Further studies over longer production periods and under health-challenge conditions are recommended to evaluate long-term impacts on growth, and potential health benefits.

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Effects of chitinase inclusion on growth performance and somatic indices of juvenile rainbow trout (*Oncorhynchus mykiss*)

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The use of insect meal instead of conventional protein sources has been shown to support fish growth and health. Insect meal is also rich in chitin, a polymer that may provide benefits to the animals. However, a high inclusion level of chitin could reduce the feed digestibility and affect fish growth and somatic status. The chitinase enzyme may help solve the problem. This study aimed to evaluate the growth performance and somatic indices of juvenile rainbow trout in response to different levels of dietary chitinase levels. A total of 480 fish (24 tanks, 20 fish/tank, 4 replicates/treatment) were fed 6 different diets containing 10% *Hermetia illucens* (HI) meal. Four of these diets were characterised by a different inclusion of chitinase (0, 0,25, 0,5 and 0,75%) obtained from *Aspergillus niger*, named CTRL, CH1, CH2 and CH3. The other two were formulated with 10% pretreated HI meal, both following a temperature treatment procedure: one was obtained without the addition of chitinase during the treatment (PRO1), while the other was obtained by adding 0,75% chitinase during the treatment (PRO2). At the end of the trial, growth performance parameters, including individual weight gain (iWG), specific growth rate (SGR), feed intake (FI), protein efficiency ratio (PER), survival rate (SR), feed conversion ratio (FCR), and final body weight (FBW), were evaluated on a tank basis. Fulton index (K) and viscerosomatic index (VSI) were also assessed on 4 fish per tank. Data were analysed by One-Way ANOVA (PER. SGR, FCR, SR, iWG, FBW, K, VSI) and Kruskal-Wallis (FI) tests ($P < 0.05$; IBM SPSS software V20.0). Trout fed CH1 showed lower FCR and higher FBW when compared to CH3 ($P < 0.05$). The CH3 and PRO1 were similar to each other ($P > 0.05$) and differed significantly from CH1 ($P < 0.01$) for PER. No differences were found for iWG, SGR, FI, SR ($P > 0.05$), as well as K and VSI ($P > 0.05$). The observed differences in the evaluated parameters may increase over the animals' entire lifespan when fed by low inclusion of chitinase in the diet. In conclusion, the inclusion of chitinase and the pre-treatment of the HI meal in juvenile rainbow trout did not lead to an improvement when compared to the control diet, and high inclusion levels seem to reduce performance. Acknowledgement: The HiGa project is supported within the Green Era Hub call by the Green Development and Demonstration Programme, GUDP, under the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF). CUP: D23C23000570005

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Dietary effects of graded levels of cricket meal on growth and gut health of Gilthead seabream (*Sparus aurata* L.)

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The effect of diets in which conventional protein sources were replaced (10, 15 and 20%) with house cricket (*Acheta domesticus*) meal (CM10, CM20 and CM30) was tested against a control diet (CM0) on juveniles gilthead seabream (*Sparus aurata*). Fish (n = 360, initial weight = 16.61 ± 0.5 g) were randomly divided in 12 tanks (n = 30) and fed the experimental diets over a 78 day period. At the end of the feeding trial, fish were measured and weighted, and viscera and peri-visceral fat were also isolated and weighted for the evaluation of somatic indexes. Intestine was sampled and processed for histological analyses. Results showed that all the experimental groups have more than tripled their body weight at the end of the trial (71.02 ± 1.85, 71.91 ± 1.96, 71.45 ± 0.92 and 70.42 ± 1.52 g in CM0, CM10, CM20 and CM30 groups, respectively). Specific growth rate was significantly lowered in CM20 and CM30 groups, compared to CM0 and CM10 ones. However, even if an equal weight gain was achieved in all groups, fish from CM10, CM20 and CM30 groups exhibited a lower viscero-somatic index compared to CM0. This result suggests a preponderant allocation of energy in muscle, thus fillet, with respect to the peritoneal cavity. Histological analyses showed overall gut healthy conditions in all the experimental groups. Remarkably, increased villi height and improved gut conditions were observed in CM20 and CM30 groups as revealed by a reduced enteritis score. Overall, the present study demonstrated for the first time the suitability of using CM in gilthead seabream diet. The present work has been funded by the Italian Ministry of University and Research, CUPB83C22002930006, Project title “National Biodiversity Future Center-NBFC.

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Black soldier fly meal as an alternative protein source in diets for largemouth bass (*Micropterus salmoides*)

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The study investigated the effects of BSF larvae meal inclusion in fish feed on zootechnical parameters in largemouth bass (*Micropterus salmoides*). A carbon footprint assessment was conducted to evaluate environmental sustainability of diets. A total of 400 juvenile fish (70.7 ± 17.9 g; mixed sex) were randomly allocated to nine tanks in a recirculating aquaculture system, (three replicate per diet; 50 fish per tank) and fed for five months with three diets: a commercial control diet (Diet A) and two experimental diets: Diet B included 15% BSF meal as a partial replacement for conventional protein sources, whereas Diet C had a similar formulation without BSF meal. The experimental diets were isoenergetic and isoproteic. Growth, feed utilisation, biometric indices, carcass and fillet traits, proximate composition, fatty acid (FA) profile, and carbon footprint were evaluated. Fish fed experimental diets B and C showed significantly greater length increase and growth than those fed control diet A ($p < 0.001$), with no differences between B and C. Feed conversion ratio was lower ($p = 0.002$) and specific growth rate higher ($p < 0.001$) in diets B and C. Condition factor increased only in fish fed diet C ($p < 0.05$), and hepatosomatic index was lower in this group than in diet A ($p < 0.01$). Carcass yield was higher in fish fed diet A ($p < 0.05$), whereas fillet yield was higher in fish fed diets B and C ($p < 0.05$). Fillets from diet B had higher lipid content. Monounsaturated fatty acids predominated across treatments, but diets B and C increased omega-3 and reduced omega-6 FAs relative to diet A, significantly improving the omega-3/omega-6 ratio. Diet B showed a reduced carbon footprint (2.09 kg CO_2 eq. kg feed) compared to diet C (2.66 kg CO_2 eq. kg feed). The substitution of soy products (soybean meal and soy protein concentrate) with BSF meal appear to be beneficial from an environmental point of view. Overall, the experimental diets improved growth performance and fillet FA quality in juvenile largemouth bass compared to the commercial diet. However, follow-up analysis will further investigate the physiological effects of BSF replacement on the immune system, tissue health and microbiota composition. From an environmental point of view, the substitution of vegetable-based ingredients (especially soy-based ones) reduces the carbon footprint of the diet by approx. 20%, suggesting a positive environmental effect of BSF meal use in aquafeed.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

The InsectFish project. The use of insect meal in the fish sector: creating value from farm to fork

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The "InsectFish project. The use of insect meal in the fish sector: creating value from farm to fork" (2023–2026) has been a multidisciplinary research initiative funded under the Italian National Recovery and Resilience Plan (PNRR) with support from the European Union – NextGenerationEU. The project was coordinated by the Department of Food and Drug at the University of Parma and had as academic partner the Department of Veterinary Sciences of the University of Pisa. Its overarching objective was to enhance sustainability in aquaculture by investigating the use of insect-derived meals as alternative feed ingredients in fish production, with the aim of reducing environmental impacts and improving resource efficiency along the value chain. A core experimental activity involved rearing *Sparus aurata* (gilthead seabream) on diets partially incorporating insect meal derived from *Hermetia illucens* (black soldier fly) larvae. This study evaluated the effects of partially replacing fish meal with partially defatted black soldier fly (*Hermetia illucens*) larvae meal (10%) in gilthead seabream (*Sparus aurata*) diets. Over a two-month feeding trial, fish fed the insect-based diet were compared with a standard control diet. Fillets were analysed for proximate composition, mineral content, fatty acid profile, lipid oxidation, colour, pH and sensory properties. Results showed no significant differences in proximate composition, pH, colour, cholesterol or lipid oxidation between diets. The insect-fed fillets had higher lauric and myristic acids, reflecting the larvae's fatty acid profile, but total saturated, monounsaturated and polyunsaturated fatty acids, including EPA and DHA, were unaffected. Mineral composition showed small shifts (higher Ca, lower P, Mg, S and Zn), without safety concerns. Sensory tests revealed that trained panellists could distinguish raw fillets, but not cooked ones. Overall, partially defatted black soldier fly meal appears to be a viable, sustainable alternative to fish meal, preserving nutritional quality while slightly affecting raw fillet sensory traits. In parallel, consumer and stakeholder studies investigated attitudes, perceived barriers and drivers, and willingness to pay for insect-fed fish products, also examining the role of information in shaping acceptance and purchase intentions. These social science components provided insights into market potential and supported the development of communication strategies for sustainable aquaculture products.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Replacing palm fatty acid distillate by mealworm oil in sheep diet: 1) effects on dairy performance and blood parameters

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Using insects as feed raises great interest, but their inclusion in ruminant diets is limited in many countries due to legal constraints. However, compared with insect protein, no restriction often applies to feeding insect lipids to ruminants. Thus, insect oils may replace dietary palm fat, which is frequently used in intensive farming because it allows for high milk yields, despite the increasing concerns on the environmental impact of oil palm cultivation. This study was conducted to investigate the effects of replacing palm fatty acid distillate (PFAD) by *Tenebrio molitor* oil (TMO) in dairy sheep diet on animal performance and some blood parameters. Fourteen Assaf ewes were used in a change-over design, with 2 periods of 25 days each and 2 experimental diets: a total mixed ration supplemented with either 2% of PFAD or 2% of TMO. Individual feed intake and milk yield were recorded daily, and milk composition was analysed thrice per week. On the last 2 days of each period, body weight was recorded and blood metabolites, enzymes and oxidative status were examined. Data collected before starting the trial (days –3, –2, and –1, when the ewes were fed the same basal diet without lipid supplementation) were used as covariate for statistical analysis. Replacing PFAD by TMO decreased feed intake and milk yield by 8%, but increased milk fat content by 6%, a positive result that might have been favoured by increased plasma non-esterified fatty acid concentration (+21%). As a result, no differences were observed in the yields of milk fat and total solids, or in feed efficiency (estimated using both feed conversion ratio and residual feed intake). Body weight, and milk protein, lactose and urea contents, as well as milk somatic cell count were not affected by treatment either. Diet supplementation with TMO improved serum antioxidant capacity by 26% and increased plasma cholesterol by 13%, whereas other blood parameters (triglycerides, beta-hydroxybutyrate, urea, creatinine, total protein, calcium, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase and derivatives of reactive oxygen metabolites) showed no differences between treatments. In conclusion, the balance between some apparently negative results (e.g., reduced feed intake and milk yield) and positive findings (e.g., improved milk fat content and antioxidant status), together with the lack of variation in feed efficiency, supports the feasibility of replacing PFAD by TMO in dairy sheep diet.
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INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Replacing palm fatty acid distillate by mealworm oil in sheep diet: 2) effects on milk fatty acid profile

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Consumers, especially those in the most advanced economies, show increasingly sophisticated demands, with aspects that include the healthfulness of animal-derived food products. In this regard, feeding unsaturated lipids to dairy ruminants is an effective strategy to modulate the fatty acid (FA) composition of their milk towards a potentially healthier profile. However, the use of insect oils for this aim has been scarcely investigated. On this basis, this study was conducted to test the hypothesis that replacing palm fatty acid distillate (PFAD) by *Tenebrio molito*r oil (TMO) as an alternative feed ingredient may improve the FA profile of sheep milk. Fourteen Assaf ewes were used in a change-over design, with 2 periods of 25 days each to test the effects of a diet supplemented with either 2% of PFAD or 2% of TMO. On the last 3 days of each period, individual milk samples were collected and stored at –30 °C. A detailed FA profile of milk samples was determined by gas chromatography analysis of FA methyl esters, and peak identifications were confirmed by gas chromatography-mass spectrometry. As expected based on the FA profile of the supplements, when TMO replaced PFAD (composed of 90% of 16:0), the milk fat content of 16:0 was reduced (–23%). This positive result was accompanied by decreases in 12:0 and 14:0 (–17 and –4%, respectively), together with most other FA with 9 to 15 carbon atoms. On the contrary, TMO induced a general increase in the concentration of 18-carbon FA in milk, including the potentially health-promoting vaccenic acid (*trans*-11 18:1; +97%), rumenic acid (*cis*-9 *trans*-11 conjugated linoleic acid; +70%), and oleic acid (*cis*-9 18:1; +37%). An increase in the potentially detrimental *trans*-10 18:1 was also observed with TMO (+111%), but its mean concentration in milk fat did not exceed 0.36% of total FA and the low *trans*-10/*trans*-11 18:1 ratio (on average 0.33) did not differ between treatments. A decrease in milk 18:3n-3 content (–11%) with TMO might result from a greater extension of ruminal biohydrogenation due to the unsaturated profile of this oil, whereas milk 18:2n-6 concentration was not affected by diet and results of 18:1 isomers support no apparent alteration in biohydrogenation pathways. Overall, replacing PFAD by TMO in the diet of dairy sheep induced changes that suggest a potentially healthier FA profile of milk fat.
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INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Insects in ruminant nutrition: uncovering their nutritive value, molecular profile, and methane-reducing potential

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The potential of insects as alternative feed is rising in global efforts to reduce agriculture's environmental impact and boost food security. The aim of this study was to evaluate the nutritive value, protein structure and the *in vitro* methane-reducing potential of *Hermetia illucens* larvae (BSFL) and *Schistocerca gregaria* (SG). This is the first study to test SG on in rumen vitro models. BSF (Livefood UK) larvae and SG (Livefoods Direct) were obtained from commercial suppliers, freeze dried for 72 hours, milled, then sieved (0.5mm). Proximate analysis followed standard protocols and nitrogen content was analysed by the Dumas method; crude protein was calculated by Nx4.76/Nx5.33 (BSFL/SG). Molecular spectra was collected using Attenuated Total Reflectance (ATR)-FTIR spectrometry, then measured in OMNIC 7.2 software. Vitamins A, B_2, B_{12}, D_3 and E were analysed by spectrophotometry, minerals by EDXRF, fatty acids by HPLC and amino acids by LC-MS. An *in vitro* ruminant model was used to study insect fermentation in the rumen at graded inclusions (0–100%) with soybean (SB). At 24hr, gas production was analysed; digesta was tested for fibre and molecular structure; and fluid for ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFAs). Significance was measured at $p < 0.05$. Results SG had the highest crude protein (53.04%), exceeding SB (46.77%) and BSFL (29.67%), while lipid content was higher in insects than in SB. Fibre fractions were similar between BSFL and SB but significantly higher in SG. Chitin content increased from SB (0.67%) to BSFL (5.42%) and SG (9.15%). Both insects contained higher amounts of vitamins A, B_2, B_{12}, and E than SB, while vitamin D_3 was not detected. BSFL was higher in Ca, K and Zn and SG in Fe and Mn, while SB had higher P and S content; heavy metals were not detected. Insects significantly reduced NH_3-N and decreased total gas and methane production – SG reduced methane by 93% compared to SB. Insect fibre was significantly more degradable than SB fibre. ATR-FTIR showed strong correlations with nutrient composition, indicating greatest rumen protein degradation in BSFL and least in SG. Reduced NH_3-N in BSFL and SG suggests limited rumen protein degradability, likely linked to chitin and lipid content, as supported by ATR-FTIR. Despite this, fermentation efficiency and the rumen environment was maintained. Overall, BSFL and SG, are highly nutritious and provide a sustainable alternative to SB meal in ruminant diets, reducing their environmental impact. ATR-FTIR spectroscopy allows assessing differences in the molecular structure of insect proteins, which influence their nutritive value.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Diet supplementation with chitosan from *Tenebrio molitor*: effects on *in vitro* ruminal fermentation and biohydrogenation

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The expansion of insect farming is increasing the availability of co-products beyond insect meal and oils. One such co-product is the insect exoskeleton, a source of chitosan. While dietary supplementation with crustacean chitosan has been shown to modulate ruminal fermentation and biohydrogenation, information on insect-derived chitosan remains scarce. Therefore, an *in vitro* study was conducted to evaluate the effects of diet supplementation with *Tenebrio molitor* chitosan on ruminal fermentation and biohydrogenation in sheep. A total mixed ration (forage:concentrate 50:50) was used as incubation substrate, either unsupplemented (Control) or supplemented with 1, 2 or 3% chitosan (MW = 100-150 kDa). Treatments were incubated for 20 h (4 runs) with ruminal inoculum from 3 cannulated sheep. Gas production (GP), disappearances of DM (DMD) and neutral detergent fibre (NDFD) and *in vitro* true substrate digestibility (ivTSD) were measured. Concentration of lactic acid and ammonia, and production of volatile fatty acids (VFA) were analysed, and CH_4 production was estimated from VFA. Digesta fatty acid (FA) composition was also assessed. All chitosan doses decreased GP ($P < 0.001$). Ammonia was reduced by 1% chitosan and ivTSD by 3% ($P < 0.05$), while lactic acid, DMD, NDFD and estimated CH_4 production remained unaffected by chitosan ($P > 0.10$). Total VFA production did not differ among treatments ($P > 0.10$), but lower proportion of butyrate was obtained with 3% chitosan ($P < 0.01$). The summation of isobutyrate, isovalerate, valerate and caproate acids was reduced with all chitosan doses ($P < 0.01$), which, together with the ammonia results, could indicate lower protein degradation. Results on biohydrogenation showed a reduction in *t*11-18:1 with all doses ($P < 0.001$) with no effect observed on *t*10/*t*11-18:1 ratio or *c*9,*t*11 conjugated linoleic acid ($P > 0.10$). Summations of FA supported that chitosan, regardless of the dose used, favoured a general promotion of biohydrogenation, as evidenced by the increase in total saturated FA ($P < 0.01$) and the lower proportion of total monounsaturated FA, polyunsaturated FA, and *trans* and total 18:1 ($P < 0.05$). Overall, despite reducing GP, chitosan does not decrease estimated CH_4 production and may be associated with a less healthy FA profile of the *in vitro* ruminal digesta. Except for ivTSD, most results showed no clear dose-dependent effect of chitosan. In any case, the long-term response of rumen microorganisms to *T. molitor* chitosan should be assessed (e.g., through *in vitro* trials with adapted inoculum and under *in vivo* conditions). Acknowledgements: PID2023-146979OB-I00, CSI020P23, FEDER and Tebrio Group S.L.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

The effects of black soldier fly larvae meal on methane and milk production in dairy cow diets

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Black soldier fly larvae (*Hermetia illucens*; BSFL) meal has the potential to provide cattle with a sustainable protein supplement that is not in direct competition with human foods. While previous *in vitro* studies suggest BSFL may reduce methane production (CH_4), *in vivo* studies in dairy cattle are limited. Our objective was to evaluate BSFL meal as a CH_4 mitigating feed for dairy cattle *in vivo* using measures of milk production, intake, rumen microbiota, volatile fatty acids (VFA), and gas emissions. Fourteen lactating Holstein dairy cows were enrolled in an experiment with a 2 × 2 crossover design, and fed either a control diet or a diet with 25% of the soybean meal replaced by BSFL meal. Periods lasted 14 days, with a 14 day washout period before switching diets. Milk samples were collected on the last three days of each period, and rumen fluid was collected on the last day of each period. Gas emissions data were captured using a GreenFeed system on the last five days of each period to capture diurnal Ch4 production. Data were analysed using mixed effects models with diet, a basal-state covariate, and their interaction as fixed effects, and cow and period as random effects. Bacterial community composition was assessed by 16S rRNA gene amplicon sequencing targeting the V1–V2 region and processed in QIIME 2. Alpha diversity (Observed species and Shannon diversity) and individual taxa were analysed using mixed-effects models, and beta diversity (Weighted and Unweighted UniFrac) was assessed using PERMANOVA in R. The BSFL diet reduced dry matter intake and milk fat yield by 5.7% and 4.7%, respectively, compared to the control diet ($P = 0.04$ and $P = 0.02$, respectively). No change in milk yield and other milk components (e.g., protein, lactose, MUN, somatic cells) was detected ($P > 0.05$). No treatment differences were detected for rumen microbial alpha diversity indices, VFA concentrations and proportions (e.g., acetate, propionate, and butyrate), nor CH4 production, intensity, or yield (all $P \geq 0.16$). In conclusion, under the conditions of our study, BSFL meal did not significantly reduce CH4 emissions in dairy cattle, and had no effects on measures of rumen fermentation. The differences observed in feed efficiency support the potential of BSFL meal to be a viable substitute for soybean meal in dairy diets, and warrant further investigation of its optimal feeding levels in commercial herds.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Effects of black soldier fly larvae meal on blood metabolites, milk fat profile, and nutrient digestion in dairy cows

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Black soldier fly larvae (*Hermetia illucens*; BSFL) meal is a high-protein food source with the potential to replace soybean meal in dairy cow diets. Our objective was to evaluate BSFL meal as a partial substitute for soybean and evaluate its impacts on blood metabolites, milk fatty acids profiles and nutrient digestion. Fourteen lactating Holstein dairy cows were enrolled in an experiment with a 2 × 2 crossover design, and randomly assigned to a control diet (CON) or a diet where 25% of the soybean meal was replaced with defatted BSFL meal (BSFL; 16% crude fat). Experimental periods lasted 14 days, with a 14-day washout. Milk, diet, and faecal samples were collected on the last three days of each period, and blood samples were obtained on the last day of each period. Data were analysed using mixed effects models with the fixed effects of diet and basal-state covariate, and cow and period as random effects. Milk fatty acids were analysed (65 detected) using gas chromatography/FID. Blood plasma was analysed for glucose, insulin, beta hydroxybutyrate, and free fatty acids, using colourimetric methods. Nutrient digestion was calculated using 240 h NDF as an indigestible marker in feeds and faeces. No differences were observed between BSFL and CON for plasma glucose (58.1 vs. 57.5 mg/dl; $P = 0.76$), insulin (7.47 vs. 7.84 μU/ml; $P = 0.79$), beta hydroxybutyrate (1.19 vs. 1.15 mM; $P = 0.65$) or free fatty acids (121 vs. 120 μM, $P = 0.94$). Milk fatty acid profile was virtually unchanged by BSFL, apart from iso-15:0 ($P = 0.02$) and iso17:0 ($P = 0.08$), respectively, which were both lower in BSFL, relative to CON. No differences in apparent total tract digestibility were observed for dry matter, crude protein, neutral detergent fibre, or crude fat (69, 72, 46 and 68% of dietary dry matter, respectively; all $P > 0.6$). Reductions in branched chain fatty acids in milk from cows fed BSFL may suggest effects on specific rumen microbe populations. Collectively, our data suggests that BSFL meal can be incorporated into dairy cow diets with minimal impacts on nutrient digestion, blood markers of metabolism, or milk fat composition. Our study supports the concept that black soldier fly larvae meal may be a suitable replacement to soybean meal in dairy cow nutrition.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Nutritional, functional and bioactive properties of protein isolates from edible insects for pet food applications

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The global pet food industry is undergoing rapid transformation driven by sustainability requirements, rising raw material costs, and increasing demand for nutritionally optimised and functional ingredients. Insect-derived proteins have gained significant attention as a sustainable alternative due to their efficient feed conversion, low environmental footprint, and high nutritional value, making them particularly attractive for premium and functional pet food formulations. Among insect-based ingredients, protein isolates offer improved functionality, and bioactive potential compared to whole insect meals. This study evaluated protein isolates (PI) obtained from *Acheta domesticus* (AD), *Tenebrio molitor* (TM), and *Zophobas morio* (ZM) for their applicability in pet food production. Insects were reared on wheat meal with carrots and cabbage, thermally inactivated, vacuum-dried at 70 °C, defatted with hexane, and subjected to alkaline extraction (pH 11) followed by isoelectric precipitation (pH 3). Lyophilized protein isolates were analysed for protein content (cf 5.6), amino acid composition, antioxidant activity, water- and oil-binding capacity, and antimicrobial activity. Enzymatic hydrolysis was performed to obtain protein hydrolysates (PH) and assess their bioactivity against *Escherichia coli* and *Staphylococcus epidermidis*. The highest protein content was observed in ZM PI (76.55%), followed by TM (45%) and AD (42%). Valine, leucine, isoleucine, and lysine were the dominant essential amino acids, while methionine was the limiting amino acid in all isolates. Aspartic and glutamic acids were the most abundant non-essential amino acids. All PI showed high antioxidant activity and contained low-molecular-weight proteins and peptides (<14 kDa) associated with bioactive functionality. Favourable water- and oil-binding capacities confirmed their suitability as functional pet food ingredients. Protein hydrolysates exhibited inhibitory effects against both bacterial strains, with stronger activity against *S. epidermidis*. In conclusion, insect protein isolates and their hydrolysates represent a promising sustainable and functional ingredient for the global pet food industry, while economic feasibility will determine their large-scale implementation. Acknowledgements: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Nos: 451-03-136/2025-03/200222 and 451-03-136/2025-03/200134).

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

Clinical effects of a *Tenebrio molitor*-based diet in dogs with food-responsive enteropathy: a histopathological study

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This study evaluated the histopathological effects of *Tenebrio molitor*-based diet in dogs diagnosed with FRE. with clinically diagnosed FRE were enrolled in a prospective dietary study. Endoscopic biopsies of the stomach and duodenum were collected before (day 0) and after 60 days of feeding a complete diet containing 35% *T. molitor* meal. Tissue samples were routinely processed and stained with HE. Histopathological evaluation was performed using the WSAVA gastrointestinal scoring system (scale 0–3). Significant improvement was observed in the stomach samples after the dietary intervention. Mean epithelial cell hypertrophy decreased from 1.36 ± 0.44 to 0.87 ± 0.41 ($p < 0.01$). Lamina propria fibrosis decreased from 0.64 ± 0.42 to 0.31 ± 0.37 ($p = 0.03$), and lymphoid follicle activity significantly decreased (from 0.44 ± 0.23 to 0.19 ± 0.19; $p < 0.01$). In the duodenum, a significant reduction in lamina propria inflammatory infiltrates was observed (mean score from 2.5 ± 0.5 to 1.6 ± 0.4; $p < 0.05$), accompanied by an improvement in the overall histopathological diagnosis of lymphoplasmacytic colitis (from 1.7 ± 0.5 to 1.2 ± 0.4; $p < 0.05$). A significant decrease in enterocyte hypertrophy was also observed (from 1.1 ± 0.3 to 0.7 ± 0.4; $p = 0.04$). There were no significant changes in intestinal villus architecture, crypt depth, or epithelial integrity. A *T. molitor*-based diet was associated with a measurable reduction in gastric and duodenal inflammatory lesions, suggesting a beneficial immunomodulatory effect. The lack of significant structural remodelling indicates that short-term dietary intervention primarily affects the inflammatory, rather than the architectural, components of the FRE. A diet containing *T. molitor* appears to be a clinically valuable nutritional option for dogs with FRE, leading to significant histopathological improvement of inflammatory lesions within 60 days. Longer-term studies are needed to assess full mucosal regeneration. Further studies are ongoing. Acknowledgements: The study was conducted during the Lider XII research project entitled “Development of an insect protein feed for companion animals with diet-dependent enteropathies”, financed by the NCBiR (LIDER/5/0029/ L-12/20/NCBR/2021). Funded by the Minister of Science under the “Regional Initiative of Excellence Program”.

INSECTS IN FOOD/FEED SYSTEMS AND NUTRITION

How does *Tenebrio molitor* affect gut microflora homeostasis in dogs?

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The use of insect protein in companion animal nutrition is gaining popularity, yet clinical evidence supporting its gastrointestinal safety remains limited. This study assessed the effects of a complete diet containing 35% *Tenebrio molitor* meal on the gut microbiome composition, metabolic activity, and fecal genotoxicity in dogs under real-world conditions. Fifteen clinically healthy adult dogs were enrolled in a 60-day feeding study, with fecal samples collected at baseline and after 30 and 60 days. Microbiological analyses were performed using 16S rRNA gene sequencing, and metabolic activity was assessed using fecal enzymatic assays and SCFA profiling. The genotoxic effects of fecal water were assessed using the alkaline comet assay on MDCK cells. High-throughput sequencing showed a significant increase in alpha diversity after 60 days, with Shannon index increasing from 3.8 ± 0.8 at baseline to 4.5 ± 0.6 ($p < 0.01$) and Chao1 richness from 255 ± 22 to 274 ± 24 ($p < 0.01$). Beta diversity analysis ($p = 0.02$) confirmed a distinct microbial community structure after the dietary intervention. The relative abundance of *Lactobacillus* and *Bifidobacterium* increased two- to fourfold compared with baseline ($p < 0.01$), whereas several proteolytic and potentially less beneficial taxa decreased. Mean faecal genotoxicity gradually decreased throughout the study, reaching a reduction of approximately 40% at day 60 compared with baseline ($p < 0.05$), indicating a lower genotoxic potential of intestinal metabolites. Enzymatic analyses revealed a significant decrease in β-glucosidase activity over time ($r = -0.71$, $p < 0.01$) along with a moderate increase in β-glucuronidase activity. SCFA profiles remained largely stable, with a decrease in acetate concentration and a slight increase in branched-chain fatty acid. In summary, the *T. molitor* meal diet induced dynamic but clinically beneficial changes in the canine gut ecosystem, characterized by increased microbial diversity, reduced faecal genotoxicity, and preserved fermentative balance. These findings support the clinical safety and potential gastrointestinal benefits of insect meal as a sustainable alternative in canine nutrition. Further studies are ongoing. The study was conducted during the Lider XII research project entitled "Development of an insect protein feed for companion animals with diet-dependent enteropathies", financed by the NCBiR (LIDER/5/0029/L-12/20/NCBR/2021). Funded by the Minister of Science under the "Regional Initiative of Excellence Program".

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Consumer priorities regarding sustainability and animal welfare with implications for insect farming

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Insect producers regularly promote the industry's sustainability benefits, but consumer research on conventional livestock suggests that welfare concerns may equal or exceed sustainability considerations in purchasing decisions. This systematic review of the evidence on consumer priorities is designed to anticipate potential challenges to insect agriculture's social license to operate and suggest means of mitigating them. We conducted a systematic review of peer-reviewed studies (2009–2025) reporting quantitative measures of consumer preferences for animal welfare versus environmental sustainability attributes in North America and Europe. Studies were included if they used stated preference methods (WTP, preference rankings, etc.) comparing welfare and sustainability. We extracted effect sizes, preference rankings and premium percentages, then identified common welfare concerns across species to project likely consumer responses to black soldier fly (BSF) production practices. Analysis of 12 studies across multiple livestock sectors revealed that welfare attributes consistently ranked equal to or higher than sustainability in consumer priorities. In WTP studies, welfare premiums averaged 15–25% higher than sustainability premiums (n = 5 studies). Preference ranking studies showed 58–73% of consumers rated welfare as the primary or secondary concern, compared to 42–51% for environmental impact (n = 7 studies). Consumer opposition was strongest (>75% disapproval) for practices involving: visible mutilation or acute pain, severe movement restriction, and perceived unnaturalness. These concerns emerged across diverse demographics and production systems. Consumer concern for farm animal welfare appears to increase with awareness and visibility. While insect production has not yet faced widespread welfare scrutiny, evidence from fish and cephalopod farming suggests concern broadens as novel sectors become familiar. The quantitative pattern that welfare concerns match or exceed sustainability priorities suggests that emphasizing environmental benefits alone may prove insufficient for maintaining public trust as insect farming scales and practices become more transparent. Applied to BSF production, this suggests several "social hotspots" that producers can manage, including refining processing SOPs, reducing purge periods, and either feeding or euthanizing adults post-egg lay. Systematic review evidence indicates welfare considerations may become increasingly relevant to social license for insect agriculture. Proactively addressing the most visible and emotionally salient welfare issues represents a strategic investment in industry legitimacy, particularly as consumer awareness grows.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

How snack processing and children's psychological profiles affect liking of insect-based snack

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Acceptance(JM1) of insect-based foods remains a major challenge for promoting sustainable diets, particularly among European children. While incorporating insects into familiar, highly processed foods generally improves willingness to try by shaping hedonic expectations (HE), acceptance varies between individuals. This variability suggests that psychological traits such as food neophobia (FN), food disgust (FD), and entomophagy awareness (EA) may influence the effect of processing level and visual cues prior to tasting. Little is known about how multiple psychological traits jointly shape children's expectations, particularly in relation to images of insect-based food that differ in processing level. Addressing this gap is critical, as early experiences may influence long-term food acceptance. This study aims to identify distinct psychological profiles among Italian primary school children (8–10 years old) and to examine how these profiles shape the interpretation of images of insect-based snacks (protein bars vs. chocolate snacks), sensitivity to processing level, and willingness to eat (WTE) such products using in-person lecture-based learning. The intervention with children (n = 105) was composed of one interactive lecture on the importance of health and environmental benefits of edible insects and a question-and-answer session with designated questionnaires. The FN, FD, EA, WTE and HE regarding images of protein bars and chocolates containing buffalo worm and cricket were measured. Data analysis included cumulative link mixed models and latent class analysis. A 3-class profiles provided the best with class distributions (41, 22 and 37%). Class membership was significantly associated with FN, FD, and gender, but not EA. Class 1 "Avoidant and disgust-sensitive" had high FN and FD, mostly females, and the lowest HE across snack types. Class 2 "Cautious but open-minded eaters" showed moderate FN and FD, mostly males, higher EF, and neutral-to-moderate HE. Class 3 "Adventurous eaters" had low FN, moderate FD, slightly more females, and the highest HE. CLMM confirmed significant differences in HE between classes for both protein bars and chocolate snacks. These findings indicate that children's acceptance of insect-based snacks depends on both psychological profiles and product type. Interventions to promote entomophagy in children should consider these individual differences and the processing of insect-based foods to effectively enhance willingness to try.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Italian consumers' perspectives on dairy and meat products from insect-fed ruminants

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Several EU studies have explored consumers' knowledge, attitudes and perceptions regarding the use of insects as feed, focusing mainly on monogastrics, while evidence on ruminants is scarce due to the EU restrictive framework, prohibiting insect proteins while allowing insect oils in ruminant feeding. This study investigates Italian consumers' knowledge of, and willingness to accept, meat and dairy products from ruminants fed insect-based diets. A questionnaire was spread across Italy, collecting responses from 1040 individuals and covering consumption habits, attitudes toward insect-based feed, sustainability, benefits and risks, knowledge of EU legislation, and socio-demographic features. Data from attitudinal sections were analysed using PCA and cluster analysis. The PCA (explained variance = 90%) identified three principal components and allowed the classification of four consumer segments: "Moderate rejectors" (41%), "Sustainability-driven" (38%), "Insect-based feed supporters" (12%) and "Purpose-driven" consumers (9%). "Moderate rejectors" showed the highest scepticism and lowest perceived benefits and were generally older and less educated, while "Sustainability-driven" consumers — typically young, urban and highly educated — strongly endorsed the sustainability potential of insect-based feeds but expressed hesitation toward their application for ruminants. "Insect-based feed supporters" displayed high acceptance despite weaker sustainability beliefs, whereas "Purpose-driven" consumers supported insect feed use in ruminants while rejecting both its sustainability framing and broader application in animal nutrition. Across clusters, perceptions of biological, chemical and allergenic risks were similar, suggesting that risk perception represents a shared cognitive barrier. Perceived positive externalities at product level (e.g., taste, safety and sensory attributes) received limited consensus, highlighting the importance of transparent, verifiable information and certification mechanisms to support consumer trust. Our findings reveal a heterogeneous and segmented landscape of Italian consumer attitudes toward insect-based feed for ruminants and provide insights for targeted communication strategies, policy development and decision-making for more sustainable ruminant production systems.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

A survey of Australian insect producers: state of the industry with a focus on biosecurity

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The insects for food and feed (IFF) industry in Australia is predicted to grow, increasing the market for insects and insect products. Biosecurity guidelines are important for the industry to scale sustainably, avoiding colony collapses and minimising risks from spillover effects. Currently, domestic and international trade of live insect or insect products in Australia is limited. However, little is known about the trade of live insect products that does occur. Introductions of diseased insect stock and improper handling could contribute to cases of pathogen transfer and insect escapes. Without knowledge of on-ground practices by producers, it is difficult to assess risk to or from the industry. We developed a survey with the aim of getting a general understanding of the state of the industry in Australia. The proposed survey has 35 questions, with 9 main themes. Two themes, 'Insect Health' and 'Biosecurity Risks' will provide industry inputs to compare with literature findings on pathogen and invasion risks. Knowledge of the prevalence and impact of pests and pathogens in commercial facilities can help prioritise future academic research. Questions relating to insect source and facility information will improve our understanding of the state of the industry in Australia and provide important context to assess risks to biosecurity. While a section of IFF literature has looked at consumer acceptance using surveys, there are few examples of surveys of producers. There is anecdotal evidence of incongruencies between industry and academic results contributing to distrust from producers. Stakeholder involvement from this research can improve trust and increase implementation of best practice strategies. Understanding on-ground practices, priorities and obstacles directly from producers can inform future research and legislative frameworks.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Multivariate sensory analysis of biscuits fortified with edible insect flours (*H. illucens, G. belina, M. subhyalinus*)

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Edible insect flours offer nutritional advantages, but their successful application depends on preserving consumer acceptability. This study evaluated the influence of insect species and inclusion level on the sensory profile of fortified biscuits. Biscuits were prepared with *Hermetia illucens*, *Gonimbrasia belina*, or *Macrotermes subhyalinus* flours at 5, 10, 15 and 20% substitution. Sensory attributes were evaluated by 51 untrained panellists. Two-way ANOVA and multivariate analyses were conducted at $p < 0.05$. The inclusion level significantly reduced sensory scores across all attributes ($p < 0.001$), with overall acceptability decreasing markedly above a 10% substitution. Insect species affected appearance ($p = 0.042$), colour ($p = 0.028$), and aroma ($p = 0.016$), but not overall acceptability ($p = 0.980$). Taste and texture were influenced solely by inclusion level ($p < 0.001$ and $p = 0.036$). No interaction effects between insect species and inclusion level were observed ($p > 0.40$). PCA explained 73% of the total variance and highlighted visual attributes as primary drivers of differentiation. CDA confirmed species-specific sensory fingerprints, while HCA demonstrated that taste and texture clustered most closely with overall acceptability. Consumer response to insect-fortified biscuits is primarily concentration dependent. A 10% inclusion level consistently preserves sensory quality across insect species, providing a statistically robust formulation guideline for product development.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Regulatory framework for insect farming in the European Union: NGO perspective

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In the last decade, the European regulatory framework governing the insect farming for food and feed sector has drastically changed, with the authorisation of insect products as novel food, animal feed, and pet food. However, this framework remains uncompleted. Eurogroup for Animals, the federation of animal protection organisations in the EU, has been reporting on insect farming in EU regulations, and has identified three crucial limitations that impedes its development. The first limitation stems from the European institutions approach of the sector through silos. Regulatory changes are asked for and considered independently, rather than by taking a comprehensive look at insect farming from substrate to the end products. There remains a lack of common understanding of what the insect farming sector should become amongst policymakers, but also in the agrifood chain. While some stakeholders push for a fully circular sector, from food waste upcycling to frass valorisation, others focus on protein production and optimisation at the expense of sustainability. Competing business models and a lack of clarity on economic viability and environmental benefits prevent policymakers and stakeholders from building a clear vision of the sector, and from advancing the regulatory framework towards it. The second limitation stems from the intrinsic complexity of the sector, itself the product of the diversity of farmed insect species, product uses, and biological constraints. The European Union general farm animal rules already miss out on species-specific regulations. Addressing the insect needs and farming conditions of holometabolous and hemimetabolous species, destined for food, feed or pet food, all under the same regulatory framework, is bound to make it difficult to evolve. Furthermore, the diversity in farmed insect species translates into a difficulty to define and address species-specific regulations related to rearing conditions, biosecurity risks, and animal welfare. Finally, the third limitation comes from the relative novelty of the insect farming sector, and of its academic field, that weakens its regulatory asks. Studies are lacking to back the development of key rules, from insect welfare needs to new substrates, in what the European Commission experts describe as an "overall lack of knowledge". Frequent assumptions or extrapolation of insect capabilities and environmental benefits that are still debated also hinder the credibility of the sector towards European institutions that remain science-driven. Fortunately, there are ways for the insect farming sector to bridge these limitations, notably by bringing forward a common, focused, and science-backed vision of what the insect farming is here for, and what benefits it can bring to the EU agricultural sector.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

From tradition to innovation: exploring entomophagy in Belgium and Benin

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Consumption of edible insects is increasingly seen as a sustainable solution to global food security and environmental challenges. Although entomophagy is deeply rooted and widely accepted in many regions of Africa, Asia, and South America, it remains largely unfamiliar and often stigmatised in Western societies. This study examined cultural perceptions and acceptance of insect-based foods among young adults (18–25 years) in Benin, where entomophagy is part of traditional dietary practices, and Belgium, where it is not commonly practiced. A comparative cross-cultural approach was employed using structured tasting events combined with standardised questionnaires. Participants (N = 135 in Benin and N = 135 in Belgium) evaluated different insect-based food products, containing whole insects (tomato sauce, muffins) and insect-derived powders (pancakes). Quantitative data were collected using KoBoCollect and analysed using a neural network implemented in R software. Results revealed marked cultural differences in acceptance of insect-based foods between the countries. In particular, participants in Benin expressed neutral to high acceptance, reflecting cultural familiarity and prior exposure to entomophagy. In contrast, participants in Belgium reported significantly higher levels of disgust, particularly towards food products containing whole insects. Nevertheless, participants in both countries expressed curiosity and openness towards tasted food products, indicating potential for attitude change. Food format also influenced acceptability, with products containing insect powders preferred over those with whole insects, especially among Belgian participants. Sustainability aspects were major motives for Belgian participants to adopt insect-based food products, while nutritional benefits – for participants in Benin. Overall, these findings demonstrate that acceptance of insect-based foods is strongly influenced by cultural background, perceived benefits, and product design. This study underscores the importance of tailored education and strategic product design in enhancing the acceptance of insect-based foods.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Traditional food culture and its relationship to entomophagy acceptance

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One of the main barriers to the adoption of insects as food in Western countries is consumer rejection, with studies reporting lower acceptance in Southern Europe compared to Central/Northern Europe. These differences may be explained by social/cultural norms, particularly the strong connection of Southern European countries to their traditional food culture. However, no studies have examined how consumers' attachment to and acceptance of their national food traditions influence their acceptance of edible insects. Moreover, given the role of disgust in entomophagy rejection, it is relevant to explore whether familiarity with traditional foods or meals that include disgusting cues is associated with acceptance of edible insects. To address this knowledge gap, a web-based survey was conducted in Portugal (380 valid answers), comprising four sections. Firstly, participants reported consumption and liking of 13 traditional foods/meals with disgusting cues. Afterwards, consumers were characterised based on their food choice motives and behaviours, attitudes and acceptance towards products incorporating edible insects (over 7-point anchored scales), and sociodemographic characteristics. Generally, participants exhibited low previous experience with entomophagy (17.6%) and acceptance of the different entomophagy forms, with unprocessed insects presenting a 21.6% acceptance rate (acceptance scores ≥5) and insect-based food products presenting acceptance rates between 36.1% ('protein concentrate') and 38.2% ('brownies'). Participants were divided into two clusters based on their level of consumption of traditional foods/meals with disgusting cues: Acceptors (n = 242) and Rejectors (n = 131). Regarding food choice motives and behaviours, the Acceptors clusters presented significantly higher scores for 'Attitudes toward traditional eating' (5.0 vs 4.6) and interest in the naturalness of their food choices (5.4 vs 4.8), while the Rejectors cluster exhibited higher food neophobia (3.9 vs 3.0) and disgust (4.8 vs 4.1). In terms of attitudes and acceptance of edible insects, the Acceptors cluster showed significantly greater acceptance of the different entomophagy forms (26.9% for whole insects and 40.9 – 43.9% for processed insects), as well as higher interest (4.4 vs 3.6) and lower disgust toward insects (3.3 vs 3.7). Lastly, the Acceptors cluster presented a significantly higher age. Consumers who consume more traditional foods with disgusting cues show greater openness to edible insects, suggesting that attachment to traditional food culture does not hinder acceptance of entomophagy. This study also highlights the role of disgust sensitivity and food neophobia in shaping consumer acceptance of foods with disgusting cues.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Tourism and gastronomy as levers for edible insect acceptability

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In the context of global food transition and environmental urgency, edible insects are increasingly promoted as sustainable and nutrient-rich alternatives to conventional proteins. However, in Europe, North America, and Australia, their adoption remains limited due to enduring cultural, symbolic, and psychological barriers. This article presents a narrative literature review exploring how experiential contexts — particularly tourism, gastronomy and hospitality — act as cultural mediators of entomophagy. Drawing on the concept of liminal micro-foodscapes, these settings are analysed as temporary spaces in which dominant norms of edibility are loosened and renegotiated. By integrating perspectives from foodscape theory, practice theory, symbolic interactionism, and gastropolitics, the article shows how insects are reconfigured through processes of aestheticisation, de-animalisation and performative mediation, enabling sensory and symbolic transformations. Comparative examples from Asia, Africa, Australia, Europe, and the Americas illustrate the diversity of entomotourism practices, from street food and community-based initiatives to educational events and haute cuisine. The discussion highlights both the transformative potential and the ambivalence of these experiences, emphasising the role of cultural intermediaries and the gastropolitical tensions involved, including cultural appropriation, unequal value capture, and selective legitimisation. Finally, the article proposes methodological contributions, including a typology of entomo-gastronomic experiences and the integration of behavioural frameworks such as the Theory of Planned Behaviour. It argues that the acceptability of edible insects cannot rely solely on sustainability or nutrition narratives but is primarily constructed within experiential contexts, where insects become "edible" through culinary staging, narrative framing, and trust in professional mediators.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Sensory analysis of cold-smoked trout fillets from fish fed insect and poultry by-products meals

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For today's consumers, palatability is the main factor influencing food choice, while other quality attributes, such as nutritional value and wholesomeness, are considered secondary. This study evaluated the effects of three experimental aquafeeds on the sensory characteristics of cold-smoked rainbow trout fillets. Fish were fed extruded isonitrogenous, isolipidic and isoenergetic diets: a control diet (C) with high fish meal (FM) content (27.3%) and two experimental diets in which FM was partially replaced with *Hermetia illucens* (HI) meal (10.1% (FM = 11.6%)) and poultry by-products (PB) meal (14.8% (FM = 11.7%)). At the end of the trial, fish were sacrificed by percussive stunning, filleted, and salted for 16 h at 3 °C. After that, fillets were rinsed, dried for 1 h at 22 °C, and cold-smoked for 3 h at the same temperature. The fillets were then vacuum packaged and stored at 4 °C. A sensory panel of 116 untrained assessors was recruited. Participants were asked to rate the overall liking and the liking of colour, flavour, tenderness and juiciness of each sample based on the 9-point hedonic scale. The appropriateness of these traits was assessed using the 5-point just-about-right (JAR) scale. Normality of the hedonic data of sensory attributes was checked by the Shapiro-Wilk test, then the non-parametric Friedman's test and the Nemenyi's post-hoc test were applied. JAR data were combined with the overall liking scores and analysed using penalty analysis to identify the sensory attributes that negatively influenced consumer preferences. No significant differences were observed among liking scores for colour, flavour, tenderness, and juiciness: fillets from all the diets were well appreciated, with mean scores above 6. The PB fillets showed the highest JAR frequencies for colour (54%), tenderness (65%) and juiciness (53%), while the highest JAR for flavour (48%) was observed in the C group. The highest frequencies for "too tender/too juicy" were observed for C fillets (25–26%), while the HI ones had the highest selection for being "too tough/too dry" (23–34%). Flavour was selected to be "too bland" for the PB fillets (24%) and colour was selected to be "too light" in C group (32%), while "too intense flavour/too dark" was identified for the HI fillets (47–44%). Penalty analysis showed that flavour was the most critical attribute, with over 20% of participants penalizing fish from all diets — especially the HI group — for having a "too intense flavour". This could be related to diet-induced changes in the fillet fatty acid composition, which may result in a more intense flavour in fish fed insect meal.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Dairy consumer and producer acceptance to black soldier fly larvae meal in dairy cow diets

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Despite the high potential of black soldier fly larvae (*Hermetia illucens*; BSFL) meal as a feed ingredient in dairy cattle, research on social perception and acceptability is limited among key dairy stakeholders, such as dairy producers and consumers. Dairy cattle rations are critical to herd efficiency and farm profitability for dairy producers. Additionally, dairy consumers have purchasing power that can lead to long-term acceptance or rejection of novel feed ingredients. Our objectives were to assess acceptance, perceived benefits and risks, potential marketing strategies, and economic implications related to the inclusion of BSFL in dairy cattle diets among (1) dairy producers and (2) dairy consumers. For objective 1, 86 dairy producers in Maryland and Pennsylvania were surveyed on familiarity with BSFL meal, comfort with its inclusion in dairy rations, perceived benefits and risks, economic adoption thresholds, and implementation support needs. For objective 2, 513 dairy consumers not restricted by state were surveyed on their background knowledge of insects and dairy rations, comfort levels, willingness to pay, marketing preferences, and demographics. Data were analyzed using mixed methods to capture quantitative and qualitative features of the data. Preliminary results indicate greater resistance to BSFL meal in dairy rations among dairy producers than among consumers. Producers displayed primary concerns related to cost and consumer response. In contrast, consumers displayed minimal understanding of dairy cattle diets and, in some cases, were willing to pay 10 – 20% more for milk from cows fed BSFL meal if perceived as higher quality and more environmentally sustainable. When asked what resources would be required to adopt BSFL meal in their dairy rations, farmers highlighted the need for success stories from other farmers, and access to a reliable supply of BSFL meal. These findings highlight a disconnect between consumer and producer perceptions of BSFL meal in dairy cattle feed. In our presentation, we discuss how farmer and consumer demographics influence interest in BSFL meal, as well as consumer preferences when purchasing dairy products from cows fed diets with or without BSFL meal. These data can inform targeted extension efforts, marketing strategies, and effective policy to support the implementation of BSFL meal in dairy cattle rations.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Gendered influence on the extent and adoption speed of cricket farming among smallholders in East Africa

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The adoption of novel foods, such as edible insects, by smallholder farmers in East Africa is emerging as an innovative strategy to enhance food and nutrition security while diversifying income sources. However, gender disparities persist due to a complex interplay of demographic, socioeconomic, psychological, and knowledge-based factors. This study surveyed 644 farming households in Western Kenya and the Masaka district in Uganda through structured interviews. Probit truncated negative-binomial, and Cox proportional hazards models examined how male versus female decision-makers influence cricket farming outcomes. Results indicated that male decision-makers adopt cricket farming more rapidly, fully embracing the practice by the second year, while female decision-makers produced 20% more crickets than their male counterparts. Experience in livestock farming and access to intensive training both significantly accelerate adoption. Conversely, the availability of training was associated with a 30% reduction in production. While access to credit did not affect adoption timing, it was linked to a 65% increase in yield. Additionally, mixed species rearing resulted in a 47% decrease in output compared to exclusive house cricket farming. Ugandan households exhibited slower speed adoption and lower productivity compared to their Kenyan counterparts. The study highlighted significant gender disparities in both adoption speed and quantity harvested. These findings underscore the need for gender-responsive policies, incentives, and context-specific training programs to address specific needs, enhance production, and accelerate adoption speed, ultimately contributing to regional food security and economic resilience.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Are insects relevant in children's diet? Factors affecting acceptance, and nutritional and health benefits or risks

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Insects have only recently entered modern food systems enabled by scaling of insect farming which can increase their availability and potential contribution to sustainable and healthy diets. To establish the relevance of insects in children's diets, evidence is needed on the complex factors influencing acceptance; contributions to nutritional gaps and any potential health benefits or risks. We conducted a comprehensive review of peer-revied publications from 2022 and 2023 addressing any aspect of the relevance of insects in children diets, with the aim of assessing the current evidence and informing research gaps. Given the very limited number of relevant publications, we applied a pragmatic search strategy. We identified 21 studies related to general nutritional quality and/or health aspects, 7 studies addressing children's acceptability, and four reviews of earlier studies. For comparison, +250 articles on dairy consumption in children and +200 on dairy-related health outcomes were published in the same period. Although more studies on insects have been published since. the evidence for the role and relevance in children's diets remains limited. For insects to be relevant in children's diet, they must be acceptable to consume. Across cultures, studies show that insects elicit both curiosity and disgust. Perception and acceptance tested through hypothetic or a single exposures in an experimental setting differed markedly from responses when exposed in familiar real-life environments. The nutritional contribution depends on the exposure, defined by the insect species and quantity consumed, as well as the broader dietary context. Studies assessing insect protein quality in animals and in human adults show variation between insect species. Standardized methods indicate that insects generally provide good protein, though slightly lower than meat and dairy, whereas biological response measures suggest comparable quality. For micronutrients, several species are recognized as rich sources of iron and zinc. The limited evidence on bioavailability shows that zinc is highly absorbable, in contrast to iron which in some species appears to be less absorbable. The potential health benefits of insects studied in humans include studies of the impacts on gut microbiota and integrity. Intestinal health is a key factor for nutrient absorption and further confirmation of the potential benefits on gut health is important. Allergy remains a key risk factor to be further investigated. Well-designed research with emphasis on human studies is needed to further understand how various insect species can contribute to improving children's diets.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Beyond simple sensory evaluation: a two-stage fuzzy logic and machine learning approach for mealworm-fortified foods

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To address global food security challenges, edible insects such as mealworms have been proposed as a highly sustainable protein source. However, low consumer acceptance remains a major barrier to their widespread adoption. This study introduces a novel Consumer Acceptance Score (CAS) system designed to move beyond traditional, one-dimensional sensory evaluation methods. We developed an integrated prediction model using Mamdani fuzzy logic and various machine learning (ML) algorithms. The model utilises multi-attribute sensory data collected from a consumer panel assessing four distinct mealworm-fortified products: rice bread, wheat noodles, rice snacks and tofu. These sensory inputs were combined with manufacturing parameters, including mealworm content and cooking methods. A two-stage fuzzy logic model was first employed to synthesize complex sensory attributes into a standardized CAS ranging from 0 to 100 points. Subsequently, four ML algorithms — linear regression (LR), decision tree (DT), random forest (RF), and XGBoost (XGB) — were applied to predict the CAS. Sensory evaluation results revealed that noodles were the most acceptable product across all age groups, with elderly consumers showing significantly higher acceptance of insect-fortified foods compared to younger participants. Statistical analysis confirmed that the CAS serves as a multidimensional indicator, showing stronger correlations with overall liking and purchase intent (r = 0.2–0.4) than individual sensory attributes alone. Among the ML models, DT and XGB demonstrated exceptional predictive accuracy, achieving R-squared values greater than 0.90, significantly outperforming the LR model. In conclusion, the proposed CAS system provides a robust and generalizable framework for predicting consumer response to insect-based foods. By integrating fuzzy logic with high-performance machine learning, this research offers a data-driven tool for food scientists to optimise processing conditions and accelerate the market integration of sustainable insect-based proteins.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Italian consumers' preferences for insect-fed Gilthead seabream: Insights from a discrete choice experiment

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Insect-based feed represents a sustainable alternative to conventional fish meal in aquaculture, offering environmental benefits and improved circularity. However, consumer acceptance remains a critical barrier to market adoption, particularly in Western societies where entomophagy is unfamiliar. This study investigates Italian consumers' preferences for farmed gilthead seabream fillets using a discrete choice experiment (DCE) that examines how information provision and product attributes influence purchasing decisions and willingness to pay (WTP). The DCE was conducted with a sample of 1112 respondents who were asked to choose among 250 g fillet alternatives differing in feed type (presence/absence of insect-based meal), the presence of a sustainability logo, a nutritional claim ("Rich in Omega 3"), and price (three levels of prices), with a no-purchase option included. The sample was divided into two groups: a control group without any information about the use of insects as feed in aquaculture, and a treatment group that was shown an informational video. The video explained that most fish we consume is from aquaculture, that currently fishmeal is used as an ingredient and this threatens marine ecosystems. In summary, the video emphasizes that insects could offer a sustainable and nutritious alternative, especially considering that many fish already consume them in nature. The aim was to analyse whether providing information on this feed innovation could impact their choices. Results indicate a strong positive WTP for both the sustainability logo and the nutritional claim, while the use of insect-based feed is, on average, negatively valued. As expected, price and the opt-out option significantly reduce utility. Interaction effects show that exposure to the informational video significantly increases WTP for insect-based feed, whereas no statistically significant differences emerge for sustainability and nutritional attributes. Overall, the findings suggest that targeted information can enhance consumer acceptance of innovative aquafeed solutions, while sustainability certifications and nutritional claims remain key drivers of perceived product value. Further analysis will employ a latent class model to identify consumer segments and explore how demographics, food neophobia, and health and eco-concern attitudes influence choices and willingness to pay.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Two decades of Dutch public–private collaboration to scale the insect sector: transition dynamics and lessons learned

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Since 2006, insects as food and feed have developed in The Netherlands from a culturally implausible concept into an internationally recognised protein innovation domain. This contribution examines how insect-based innovation can transition from a protected niche to a resilient and scalable practice. Drawing on two decades of experience, it reflects on lessons from the Dutch Diamond approach, characterised by structured collaboration between research, government, and the private sector. Grounded in innovation and sustainability transition theory and informed by the Multi-Level Perspective (MLP), the Dutch insect sector is analysed as a niche innovation interacting with established food, feed, and regulatory regimes. A persistent constraint has been the misalignment between Technology Readiness Levels (TRL) and Societal Readiness Levels (SRL). While breeding, production, and processing technologies advanced relatively quickly, societal acceptance, regulatory adaptation, and market uptake progressed more slowly, limiting scale-up. Within the Dutch Diamond approach, Wageningen University & Research serves as an independent knowledge anchor. At a time when insects for human consumption were widely considered unrealistic, it provided scientific credibility and continuity, enabling evidence-based dialogue across science, policy, and society. In parallel, the Dutch Ministry of Agriculture, Nature and Food Quality supported regulatory learning and adaptive governance, allowing experimentation while safeguarding food safety and public trust. Entrepreneurs shaped sectoral roadmaps and co-invested through public–private partnerships aligned with national insect and circular agriculture agendas. Early expectations were often optimistic, underestimating the time and coordination required to align societal readiness with technological progress. The establishment of the Dutch Association of Insect Producers (VENIK) and platforms such as the Green Deal Insects for Food, Feed and Pharma and Insect Coalition NL institutionalised long-term consultation structures linking sector ambitions with research and policy. These arrangements stabilised the interaction between regulatory caution and entrepreneurial ambition. Overall, the Dutch experience shows that scaling insects for food and feed is primarily a governance and transition management challenge rather than a technological one. The Dutch Diamond approach offers a transferable model for aligning TRL and SRL through structured dialogue and adaptive policy.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Unlocking the potential of edible insects-spirulina foods: consumer perception, acceptance and nutritional enhancement

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The consumption of insects (entomophagy) has gained attention in Western diets as a sustainable alternative to conventional protein sources. Spirulina, a widely cultivated cyanobacteria, representing over 30% of global microalgal biomass production, is also recognised for its high nutritional value, particularly a rich protein source. In response to the growing demand for more sustainable and high-quality nutrients, a consumer acceptability survey was conducted to assess Portuguese consumers' awareness, perceptions and acceptance of insects and spirulina as food. Higher acceptance was observed when insects were incorporated in ground or flour form. Based on these results, four food products (crackers, bread, pancakes and pasta) incorporating *Tenebrio molitor* larvae flour and spirulina at different concentrations were formulated to enhance their protein content and nutritional value. The formulations were evaluated for their sensory attributes, proximate composition, amino acid and fatty acid profiles, and immunoreactivity. Sensory analysis was performed using a Check-All-That-Apply (CATA) approach, comparing formulations with respective controls. Immunoreactivity was assessed by immunoblotting and ELISA using sera from crustacean-allergic patients. The addition of insects and spirulina did not negatively affect texture attributes, although the green colour influenced acceptability, being associated with "vegetal" or "algae" characteristics. The novel products showed increased protein, amino acid and fatty acid contents without significantly altering their profiles. IgE cross-reactivity with insect proteins was observed in crustacean-allergic sera. Overall, the novel insect–spirulina supplemented products show potential as sustainable, protein-rich foods, supporting further industrial development and commercialisation. Funding: This work received financial support from European Union, FEDER, through the projects EMPOWER (COMPETE2030-FEDER-00861800). Acknowledgments: This work was also supported by the national PT funds (FCT/MECI) through the projects INSPIRE (2024.14868.PEX), ALLEVIATE (2023.12193.PEX), UID/50006/2025, and IMMUNOGATE (COMPETE2030-FEDER-00848700).

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Turning waste into wealth: harnessing insect protein for sustainable greener poultry production and consumer adoption

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This paper presents a circular supply chain using a four-step upcycling strategy of agro-industrial food waste through insect (black soldier fly (*Hermetia illucens* L.) larvae, BSFL) bioconversion. The larvae were processed into full-fat and defatted BSFL meal (FF-BSFLM and DBSFLM, respectively). The protein-rich FF-BSFLM and DBSFLM were used to substitute soybean meal (SBM) and fishmeal (FM) in Cobb500 broiler feed and impact on growth performance validated. Consumer behaviour, preferences and willingness to buy broiler meat were also established. BSFL upcycle potato waste with 1.4% crude protein to achieve biomass yield with 38.17% crude protein, 34.48g weight gain, 77.14% waste reduction and 8.55% bioconversion. Defatting BSFLM increased the crude protein levels by 50.73% (i.e., from 38.18 to 57.53%). Partial substitution of FM with DBSFLM increased daily weight gain and feed conversion ratio by 5.1–15.2 and 2.9–5.2%, respectively, at the starter and finisher phase, respectively. Feed cost significantly reduced for BSFLM-based diets with the highest marginal net benefits ($3.1) and rates of return (437.8) for 50% FM + 50% DBSFLM. There was improved flavour, juiciness, tenderness, and taste of meat from birds fed diets with 50–100% BSFLM inclusion levels (7.6–27.5%). Acceptability (88%) and willingness to buy (82%) meat was highest for consumers provided meat from birds fed diet with FF-BSFLM. These findings demonstrated that insect-based nutrient recovery was a sustainable strategy capable of addressing issues related to food waste and pollution, while promoting self-reliance in feed production through a circular economy lens.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Analysis of the digital market for edible insects in Mexico

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Mexico has 549 species of edible insects, out of nearly 1900 identified worldwide, whose consumption is part of the food tradition in rural and Indigenous communities. However, in urban contexts, these foods are often perceived as exotic or unpleasant products. In order to analyse this perception in the market, the present study identifies the main companies involved in the production and commercialization of edible insects in Mexico and examines their digital presence from the perspectives of public perception and their marketing strategies. A systematic digital search was conducted, along with an analysis of web-based content from Mexican companies dedicated to the production of insects for human consumption. Nine companies specialized in this sector were identified, considering the species produced, the product presentations, and the commercialization channels used. Their commercialization is referenced to NOM-251-SSA1-2009, as there are no specific regulations of this kind in Mexico. Nine companies focused on human consumption were identified, along with three startups producing animal feed and one oriented toward fertilisers. All of them have websites for e-commerce, which has become their main sales channel; on four websites, English is the predominant language, despite the companies being established in Mexico. The combined product offering comprises 19 products, classified as flours or powders (10), whole-insect presentations (4), snacks (1), sauces (2) and salts (2). Price ranges vary from MXN $330 to $2500 for flours, MXN $200 to 700 for whole insects, and MXN $90 to 200 for snacks, salts and sauces. Marketing strategies are primarily oriented toward the fitness sector, followed by positioning insects as snack alternatives and, to a lesser extent, an exotic culinary approach. None of the companies make explicit reference to the ancestral consumption of insects. The companies' production systems are tower-type breeding systems, with one of them relying on the exploitation of wild species. The findings highlight: (1) the development of specific sanitary and regulatory standards; (2) greater investment in culturally contextualized marketing strategies targeted at consumer segments with above-average income levels; (3) diversification of the product offering, particularly in relation to the informal market; and (4) cost reduction, as a minimum price of MXN $200 limits accessibility. All of this is aimed at fostering a favourable cultural shift toward the consumption of insects produced in controlled breeding systems.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Traditional knowledge about the consumption of edible insects: evidence from KwaZulu-Natal and Limpopo provinces

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Consumption of edible insects is an indigenous practice that has played an essential role in human nutrition across Africa. The traditional use of insects forms an important part of food culture in Africa, and insects are consumed either as a delicacy, emergency, or staple source of food. However, indigenous knowledge about insect consumption is being lost because recent generations have adopted western methods and paid less attention to traditional practices. We conducted 500 questionnaires in five local municipalities in Kwazulu-Natal (KZN), and 122 questionnaires in four local municipalities in Vhembe district in Limpopo, South Africa, to document indigenous knowledge about edible insects' consumption, collection, and preparation methods used in Limpopo and KZN. Eight insect species belonging to five insect orders were used as food in Limpopo and KZN, with mopane worms (94%) and termites (70%) being the most preferred species by respondents in Limpopo and KZN, respectively. Ninety-five percent of the respondents occasionally consumed insects in Limpopo, while only 28% did so in KZN. Nutritional benefits and tradition were the main reasons for consuming insects. Edible insects are a nutritious diet and play an important role in people's livelihoods in rural areas. However, there was a notable decline in entomophagy, particularly in KZN. The decline may be related to occidental acculturation, discomfort associated with eating insects, and declining insect availability. To promote entomophagy, the authorities should encourage people to include edible insects in their diets because of their nutritional value. In addition, edible insect flour should be incorporated in food products such as biscuits, bread, energy bars, cereal, and cookies to promote acceptability. Keywords: entomophagy, nutritional benefits, mopane worms, termites, livelihoods.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Drivers of edible insect consumption in Mexico: market and nutritional motivations

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This study analysed consumer motivations for increasing edible insect consumption frequency in Mexico. Results from the categorization of survey data, visualized through a word cloud, identify availability and price as the most significant drivers. Findings reveal that consumers prioritize competitive pricing relative to conventional proteins and better accessibility in local supermarkets. Responses highlight that while curiosity triggers initial tasting, the transition to frequent consumption is driven by Information regarding high protein content and health benefits, such as antioxidant properties. The data shows that appearance is a critical factor, with a clear preference for "invisible" formats like powders and flours to mitigate sensory rejection. Additionally, results indicate that sustainability and cultural identity are secondary but stable motivators, particularly in regions with traditional entomophagy heritage. While price sensitivity remains a primary barrier, the findings suggest that the frequency of consumption increases when nutritional value is clearly communicated alongside improved market accessibility. These results demonstrate that moving insects from a delicacy to a dietary staple requires a strategy focusing on affordability, processed formats, and targeted nutritional education.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

The role of entomological societies in developing best practice for insect farming

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As insect farming expands, the sector requires scientifically grounded frameworks that support safe, consistent and commercially resilient production. Entomological societies are well positioned to contribute to this development through their operation at the intersection of scientific research, industrial practice, and regulatory discussion. The importance of this role is reflected in the 2025 Manna Insect Industry Survey, in which producers identified the Royal Entomological Society (RES) as one of the most trusted influencers across the sector. The work presented will outline how the RES is working to support the insect as food and feed industry by facilitating the development of best practice guidance and Codes of Practice (CoPs) for farmed insects. The central aim of this work is to provide industry with clear, evidence informed tools that can strengthen production systems, improve consistency, and help prepare the sector for future regulatory expectations. A key component of the RES approach is to bring producers, scientists, regulators and other stakeholders into early and constructive dialogue, thereby ensuring that developing guidance reflects both practical realities and policy trajectories. Early initial activities include early engagement with producers to map current practices and priorities, and preparation for the first multi stakeholder roundtable, which will directly connect industry representatives, researchers, and regulatory bodies. This early engagement is designed to clarify feasibility, identify areas requiring further research, and facilitate a shared understanding of how the sector can grow in a responsible and sustained manner. By analysing and sharing insights from this preliminary stage, it is celebrated that entomological societies can provide effective institutional support for industry led development. They can help build consensus, reduce uncertainty around regulatory expectations, and promote an international landscape of coherent and scientifically credible production standards. We highlight how the RES aims to offer trusted leadership for a rapidly evolving industry that benefits from early, coordinated and evidence-based guidance.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

Willingness to adopt insect farming in Africa is associated with risk attitude and perceived benefit

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Agri-food systems in low-income countries face constraints, highlighting the potential of insect production. Globally, insect farming has grown, supported by public–private partnerships aimed at enhancing food security, nutrition, and livelihoods for smallholder farmers. However, evidence on the drivers and barriers to adoption — particularly smallholders' willingness to adopt insect farming — remains scarce. This gap hinders the development of targeted interventions to address farmers' constraints and preferences. We conduct the first rigorous investigation into smallholder farmers' willingness to adopt insect farming, using data from 8886 smallholders in Ghana, Kenya and Uganda. Our findings indicate that positive perceptions of the benefits of insect farming and low risk aversion are associated with higher willingness to adopt, while dietary diversity is negatively correlated. The results suggest heterogeneity in adoption willingness: female farmers with low risk aversion, educated farmers with low risk aversion, and those relying on informal agricultural information sources are more likely to adopt insect farming. We conclude that interventions should focus on emphasizing the benefits of insect farming and addressing risk aversion directly. Additionally, leveraging informal information channels and targeting households with low dietary diversity can further enhance adoption rates.

SOCIETY, PERCEPTION, AND CULTURAL DIMENSIONS

More than instinct: biometric analysis of emotional barriers to entomophagy using FaceReader

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Edible insects are widely regarded as one of the most promising alternative protein sources. Yet despite their nutritional and environmental benefits, strong prejudices persist in many Western societies, resulting in low willingness to consume insect-based foods. The aim of this study was to explore consumers' genuine attitudes toward entomophagy by objectively assessing subconscious emotional responses to audiovisual stimuli. Because a substantial share of affective reactions occurs automatically and outside conscious awareness, biometric methods can provide valuable insight into psychological barriers that may not be captured through self-report measures. A total of 227 participants took part in the study. Real-time emotional responses were recorded using FaceReader while participants watched an original film entitled Entomophagy. The biometric analysis focused on scenes showing: (i) insect farming (adult and larval *Tenebrio molitor* and house cricket *Acheta domesticus*) and (ii) dishes prepared with insect powder or with insects presented visibly. FaceReader classified basic emotions and computed valence and arousal indices. The strongest emotional response – reflected by the lowest proportion of neutral expression in both genders – occurred during the scene featuring adult *Tenebrio molitor*. Men showed a significantly higher and more stable level of neutral expression, indicating lower overall emotional reactivity than women. In women, sadness and anger increased significantly during the film. By contrast, although disgust was detected, changes in disgust did not reach statistical significance in either group. The lack of a pronounced increase in disgust, alongside elevated sadness and anger in women, suggests that resistance to entomophagy may be driven more by broadly negative affect and cognitive discomfort than by simple revulsion. Participants may have experienced unease arising from the tension between information about benefits and long-standing cultural beliefs. The higher neutrality observed in men may reflect lower overt expressiveness or different emotion-regulation patterns, potentially obscuring the intensity of their internal reactions. Visual exposure to adult insect forms is the key emotional barrier to accepting entomophagy. Communication strategies should therefore avoid imagery of adult specimens and instead emphasise the appealing presentation of foods made with insect powder. This approach may reduce negative emotional reactivity and support greater acceptance of insects as a food ingredient.

GLOBAL PERSPECTIVES AND APPLICATIONS

Economic feasibility of valorizing hatchery residues in Quebec through fermentation and black soldier fly bioconversion

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The management of hatchery residues (HR) poses economic challenges for producers due to the high costs associated with thermal rendering. Stabilising these residues through fermentation followed by bioconversion using black soldier fly larvae (BSFL) represents a promising and potentially cost-effective alternative. To assess the feasibility of this process, economic predictions were performed using a tool adapted to the specific BSFL production context in Quebec, to identify optimal implementation strategies. The tool integrates 32 scenarios, including centralized and decentralized models with processing capacities ranging from 10 to 110 tonnes of residues per week (tpw), two carbohydrate-rich co-substrates for fermentation (wet or liquid whey permeate), two reproduction strategies (in-house colony vs. external BSFL supply), and two marketing approaches (bulk vs. retail). Capital and operational costs were estimated for each scenario over four years, along with revenues from the sale of dried larvae for pet food and composted frass as fertiliser, allowing annual profit calculations. The analysis considered production starting at 30% of design capacity with a subsequent ramp-up of 30% per year. Results indicate that the large-scale centralised model (110 tpw) with an in-house colony is the most profitable scenario, with a maximum profit of 628 USD/t of treated HR under most favourable conditions (retail sale price and full-capacity operation). Furthermore, even without generating profit, decentralized models remain economically advantageous for hatcheries processing at least 15 tpw, as their associated losses (25 to 176 USD/t of treated HR) are lower than the cost of using rendering services to manage HR (178 USD/t). Nevertheless, the availability of qualified labor, regulatory constraints, and market competition for both inputs and products limit overall feasibility. This study provides a detailed overview of the costs, revenues and risks involved, offering valuable insights for producers and policymakers to support the adoption of alternative livestock residue management strategies.

GLOBAL PERSPECTIVES AND APPLICATIONS

A "Hub & Spoke" BSFL model in Myanmar: overview, applied R&D and market development

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Myanmar is facing a prolonged and compounding crisis marked by political upheaval, economic instability, and repeated natural disasters. Rural livelihoods are under severe strain, with farmers experiencing declining incomes, limited access to capital, and rising costs for fuel and protein-rich animal feeds. At the same time, Myanmar possesses strong enabling conditions for insect-based feed systems, including abundant agricultural and organic waste streams, a large smallholder aquaculture and livestock sector, and a tropical monsoonal climate well suited to Black Soldier Fly Larvae (BSFL) production. This paper presents and evaluates a decentralised "Hub & Spoke" BSFL production model developed, piloted, and rolled out in Myanmar by Spectrum – Sustainable Development Knowledge Network. Unlike large-scale industrial BSFL factories — which face prohibitive capital, financing, logistics, and scaling barriers in fragile economies — the Hub & Spoke model prioritises low-cost, locally adapted, and socially embedded production. Small decentralised "cells" of BSFL growers — predominantly women — are supported by local breeders and waste/resource collectors, while a central hub provides training, applied research, quality oversight, and coordination. Applied research demonstrates the feasibility of using diverse local substrates, including market waste, fish waste, invasive species (water hyacinth, pleco fish, golden apple snail) and agricultural residues such as banana pseudostems. Nutrition-based substrate design, often incorporating fermentation, has proven effective without requiring highly standardised inputs. Integrating waste collectors into production cells improves livelihoods, supports organic waste separation, and enables decentralised resource flows, turning high transport costs into a structural advantage. Both BSFL biomass and frass are utilised within integrated farming systems. Frass shows promise not only as fertiliser but also as a partial feed ingredient, simplifying processing and increasing overall protein yields. Small-scale aquaculture trials across 12 locally important fish and shrimp species demonstrate consistently positive palatability results for BSFL-based feeds. Monitoring and scaling rely on low-cost tools, citizen science and social media-enabled peer networks, demonstrating that robust data collection can occur without complex technologies. Overall, the Hub & Spoke BSFL model aligns well with Myanmar's socio-economic and environmental realities, offering pathways to improved food security, waste valorisation, environmental services and resilient rural livelihoods, with longer-term potential to supply emerging markets such as pet food and, eventually, commercial feed mills.

GLOBAL PERSPECTIVES AND APPLICATIONS

Disgust, culture and colonial legacies in attitudes toward edible insects

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Western resistance to the consumption of insects persists despite growing evidence of their nutritional adequacy, environmental sustainability, and historical role in human diets. While ecological factors initially shaped regional food practices, contemporary rejection of insects as food in Western societies appears disproportionate to empirical risk. We examine whether such resistance is best explained by ecological constraints, psychological mechanisms, or enduring colonial and eurocentric frameworks that continue to shape food norms in both Western societies and the Global South. A qualitative narrative synthesis was conducted drawing on interdisciplinary literature from anthropology, archaeology, nutrition science, environmental studies, psychology, and postcolonial theory. Evidence on early human diets, traditional insect consumption, nutritional and environmental assessments, was integrated with critical analyses of eurocentrism, disgust, and decolonization. Archaeological and ethnographic evidence indicates that insects constituted a reliable and nutritionally valuable food source throughout human evolution. Contemporary analyses show that edible insects provide high-quality protein, essential micronutrients, and lower environmental impacts than conventional livestock. In tropical regions, year-round availability explains their historical integration into food systems. In Western societies, limited ecological availability historically constrained consumption, later reinforced by food neophobia and culturally embedded disgust. In parts of the Global South, insect consumption is declining, particularly among urban and educated populations. Disgust toward insect consumption operates not only as a disease-avoidance response but also as a cultural boundary marker tied to identity, modernity, and perceived civility. Eurocentric and colonial imaginaries have framed insect-eating practices as primitive or poverty-driven, contributing to stigma and internalized devaluation of Indigenous food systems. Contemporary conspiracy narratives in Western contexts reflect anxieties about cultural change rather than objective concerns about health or safety. Resistance to insect consumption in Western societies is not grounded in nutritional, safety, or environmental evidence but is shaped by affective disgust and colonial hierarchies of knowledge. Decolonization requires recognizing Western dietary norms as culturally specific, legitimising Indigenous food systems, and addressing power relations embedded in contemporary food discourse. Without such critical reflection, efforts to promote insects as food risk reproducing colonial dynamics rather than challenging them.

GLOBAL PERSPECTIVES AND APPLICATIONS

Cultivation and welfare of the true bugs (Hemiptera) traditionally farmed for use in human food

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The true bugs, Hemiptera, constitute a hyperdiverse clade within the Insecta. Of these, several of the scale bugs (Sternorrhyncha) have been prized by humans in part due to their interesting life history: the mature females affix themselves to their host plants using a secreted resin and afterwards remain motionless for the remainder of their lives. Many species of scale bugs are harvested from the wild, but the cochineal bug (a.k.a. *cochinilla*), *Dactylopius coccus*, is also farmed industrially on plantations to produce the food dye, carmine (E 40), which serves as an economically and culturally important resource. This presentation focuses on farming and production methods for these understudied insects, covering historical and modern practices and identifying potential welfare challenges and opportunities using the Five Domains framework. Findings indicate that at least 140 trillion cochineals have been farmed throughout history, and there are many opportunities to improve insect welfare, since contrary to what would be expected, the highest quality raw cochineal can be acquired through non-lethal harvesting methods.

GLOBAL PERSPECTIVES AND APPLICATIONS

Critical business metrics of BSF bio-waste operation: comparative analysis across developed and developing countries

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Black soldier fly (*Hermetia illucens*) larvae bioconversion represents an emerging technology for bio-waste management, yet validated operational metrics across facility scales and economic contexts remain limited. This study synthesizes critical business metrics from BSF bio-waste cases from 0.5–50 tonnes/day across Europe, Africa and India, comparing developed (EU) versus developing country operations. Analysis and interviews were made for three different locations where two had BSF operations and one was planning one. All local metrics from electricity, wages, climate conditions, regulations, prices of animal feed and fertiliser were included in the analysis. CAPEX and OPEX costs were evaluated based on real references, and sales prices were compared to regionally available commercial feed and fertiliser prices. CAPEX for tn/d biowaste conversion capacity varied from $25 000 to 85 000 between developing and developed countries and optimal climate conditions. OPEX varied from $20 to 50 per biowaste tonne respectively. Profitable business, even without any tipping fees, was more likely in developing countries where regulation was more flexible on using larvae for animal feed, while frass was priced similarly regardless of country's development level. BSF based bio-waste management operation can be competitive in both developed and developing countries, but it depends on multiple critical factors which need to be analysed beforehand. This paper shows the key metrics to validate for evaluating if BSF based bio-waste operation is competitive, and case examples from developed and developing countries.

GLOBAL PERSPECTIVES AND APPLICATIONS

Estimating economic values of traits in *Hermetia illucens* production in the tropics: a bio economic modelling approach

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The black soldier fly (BSF, *Hermetia illucens*) is gaining recognition as an alternative source of protein in animal and aquaculture feeds by recycling diverse organic wastes in the Global South, where demand for sustainable and non-food-competing protein substitutes for animal feed is rapidly increasing. Despite its growing adoption in the tropical region, the economic values (EVs) of biological traits, which are essential in defining a clear breeding target, have not yet been quantified. This study developed a bio-economic model integrating biological processes with economic components to estimate the EVs of major production traits in a tropical BSF larvae production system in Uganda. The model simulated a commercial BSF production system in Uganda operating at a fixed capacity of 3750 kg of wet larvae per production cycle (12 days). The main revenues included in the model were whole and processed larval meal, fat, eggs, 5-day-old larvae (5DOL) and frass. Revenues from eggs and 5DOL are considered only from surplus production, as these products are sold after meeting the target 3750 kg wet larvae. The costs included commercial and organic waste feed, larval meal processing, housing and labour for larvae and breeding management. The EVs of 13 different traits were calculated as the marginal change in profit resulting from a 5% increase in each trait mean, while holding the level of the rest of traits fixed. The baseline profit was estimated $1542 per cycle with total returns of $3067 from multiple sources of revenue against costs of $1524. Harvest larval weight and survival from day six to harvest show the largest positive EVs ($139 each). Five-day-old larval weight also showed a strong positive EV of $79 ranking just after harvesting larval weight and survival. Compositional (larval dry matter, protein and fat percentage) and reproductive traits (egg hatchability and eggs per female) have moderate EVs ($12 to 33). Traits related to feed intake and production time have negative EVs (–$4 to –$33). In conclusion, the bioeconomic model results indicate that tropical BSF production systems are primarily driven by traits that directly enhance harvestable biomass, especially larval weight and survival rate. The estimated EVs offer a quantitative basis for sustainably improving BSF production in tropical region by identifying economically important traits and guiding the design of breeding objectives, genetic improvement and selection strategies.

GLOBAL PERSPECTIVES AND APPLICATIONS

The simplified black soldier fly approach for small-scale, part-time farming: experience from northeastern India

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While medium- to large-scale black soldier fly (BSF) farms require daily management, such a time commitment is challenging for smaller operators. This creates a need for standard operating procedures (SOPs) that enable part-time operation while still ensuring reliable and consistent production. The SIMplified BSF Approach (SIMBA) was developed as part of the SWIFT project in Malawi and has now been piloted in north eastern India (Nagaland, Assam and Manipur). SIMBA is suitable for beginners exploring BSF waste processing, for small farms producing BSF larvae as feed for chickens, pigs, or fish with limited labour availability, and for research or training centres operating small-scale units. The system is based on a schedule requiring a few hours of work on two days per week and is designed for a treatment capacity of approx. 500 kg of organic substrate per week. Three central SIMBA facilities were established in the Dimapur region as production, knowledge, and training hubs and as distribution points for young BSF larvae used to treat pig manure. Pig manure represents a management challenge for many farmers, as agricultural fields are often far from pig sheds and the manure cannot be applied directly around homesteads due to its high biological activity. Common practice is therefore to dump manure behind pig sheds, attracting vermin and increasing disease risks. The hubs are used to train interested farmers from surrounding areas in the application of SIMBA. Hub operators adapt the SIMBA SOPs to local climatic and cultural conditions before transferring this knowledge to new users. Selected farmers received starter kits for larval grow-out on pig manure. Young BSF larvae are collected bi-weekly from the hubs, distributed to the grow-out farmers and added to the manure, with larvae and frass harvested after approx. 10 days. Although pig manure shows lower conversion performance than more suitable substrates such as canteen or kitchen waste, farmers have adopted SIMBA to better manage locally generated waste streams and reduce expenditure on commercial feed for poultry and fish. Operational experience showed that system performance met or exceeded expectations during most of the year. Even during colder winter months, when night-time temperatures drop below 10 °C in December and January, continuous operation remained possible following minor SOP adaptations. As a next step, interested villages and communities will be trained in the SIMBA reproduction cycle, enabling autonomous operation beyond the training hubs.

GLOBAL PERSPECTIVES AND APPLICATIONS

Context matters: designing medium-scale BSF systems for African markets – evidence from Uganda

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Black soldier fly initiatives are implemented in a wide range of contexts and follow diverse organisational and technical approaches. In practice, many projects prioritise either low-tech waste treatment objectives or capital-intensive industrial insect protein production, often shaped by target markets and financing structures. The Bioconvision case in Jinja, Uganda highlights a deliberately different positioning. The facility is designed as a medium-scale, labour-based BSF system that balances product consistency, operational simplicity, and financial feasibility within its enabling environment, rather than maximising scale or automation. From the outset of the facility's establishment in 2024, large-scale waste treatment was deliberately postponed. This was both a technical and financial choice, reflecting limited access to capital and allowing biological stabilisation, market testing, and early revenues before committing to the full investment. Operations began with the construction of a reproduction unit sized to supply larvae for a ten-tonne-per-day facility, combined with basic infrastructure for treating 500 kg of waste per day. This enabled small-scale production and early sales of larvae while substrate logistics and market demand were tested. Only after reproduction performance and waste sourcing had stabilised was the grow-out unit built. Today, the facility processes eight tonnes of organic waste per day through contracts with market waste collectors, a brewery, a fish processing plant, and local fishermen harvesting water hyacinth. Daily outputs are approximately 960 kg of fresh larvae and 3.6 tonnes of frass. Total investment for the ten-tonne facility, including buildings, machinery, licensing, commissioning, and early operating costs, amounts to EUR 450 000. Operating costs are currently EUR 135–140 per tonne of treated substrate, while revenues are approximately EUR 155 per tonne, driven by sales of fresh larvae (around EUR 0.72/kg) and matured frass (around EUR 0.40/kg). These figures reflect an early operational phase and indicate that further margin improvement is required for long-term financial robustness, primarily through scale, improved conversion efficiencies, and gradual market development. In the Bioconvision case, the start-up phase faced several operational constraints, including unreliable electricity supply, commissioning delays, limited access to capital, and a lack of local technical expertise. The case suggests that aligning infrastructure development, contractual arrangements, and cash flow with the enabling environment can be as decisive as biological performance when establishing BSF facilities under real-world conditions.

GLOBAL PERSPECTIVES AND APPLICATIONS

Scaling black soldier fly microfarming for food security and circular agriculture: evidence from four regions, Madagascar

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In much of rural Madagascar, smallholder farmers have limited or no access to commercial animal feed, relying on household scraps, unmanaged organic waste, or seasonal foraging to support poultry, fish and crop production. Organic waste is widely available but rarely valorised, while soil fertility continues to decline. Black soldier fly (BSF; *Hermetia illucens*) microfarming offers a low-cost circular approach that does not depend on cash inputs, yet evidence from small-farm systems remains limited. We present results from a multi-site BSF small-farm programme implemented across four regions of Madagascar, integrating production, livelihood outcomes, and social acceptability. Between 2024 and 2025, BSF systems were established in 42 communities across Farafangana, Fénérive-Est, Anivorano, and one emerging site, supported through standardised training, starter kits and follow-up. Monitoring covered 234 active microfarms, including 181 newly established in 2025. Across sites, an estimated 188 065 kg of organic waste was recycled, producing 31 344 kg of BSF larvae and 135 075 kg of frass fertiliser. Among monitored farms (n = 87), mean production reached 15 kg of fresh larvae and 42 kg of frass per farm per month, with high-performing farms producing up to 73 kg of larvae monthly. The average annual economic benefit per household was estimated at MGA 859 327, reflecting substitution for unavailable or unaffordable external inputs rather than direct cash savings. Agroecological assessments indicated moderate-to-high adoption, with BSF microfarming scoring 40 – 70% on the FAO TAPE agroecological transition framework. Farmer-to-farmer exchange visits and regular follow-up supported sustained adoption, while constraints included seasonal feed availability, predator pressure and household labour limitations. Targeted cooking demonstrations and nutrition outreach increased acceptance of BSF larvae for household consumption in food-insecure regions. These results demonstrate that BSF microfarming can function as a scalable, farmer-accepted circular farming system where commercial feed markets are absent. Rather than optimising production efficiency alone, successful deployment depended on replacing missing inputs with locally available resources, aligning practices with household realities and supporting multiple on-farm uses, offering practical lessons for insect-based food security initiatives across the Global South.

GLOBAL PERSPECTIVES AND APPLICATIONS

Insect management in agri-food systems: validation of a multidimensional tool using edible species

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Insects perform multiple socio-ecological roles in agri-food systems, contributing to food production, ecosystem functioning and circular bioeconomy development while also generating context-dependent risks that require differentiated management decisions. Despite growing interest in insects as food and feed, stakeholders still lack integrative tools capable of comparing species beyond productivity alone when planning sustainable applications. Within the Insectonomy programme, a research initiative integrating ecosystem services, management strategies and socio-ecological risks of insects, we developed and refined a multidimensional assessment tool designed to support insect management across farming, wild gathering, conservation, and pest and vector control contexts. The framework integrates indicators of productive potential, ecosystem contributions, current and prospective uses, and biological, technological and governance-related challenges. The tool was first applied to a comparative dataset of 120 insect species representing farmed, harvested and conserved taxa, combining harmonised qualitative scoring with multivariate analyses including principal component analysis, UMAP ordination and clustering to identify functional groups linked to distinct management strategies rather than single optimal species. To examine relevance for food and feed systems, the tool was subsequently validated using six edible species *Hermetia illucens*, *Tenebrio molitor*, *Acheta domesticus*, *Zophobas atratus*, *Alphitobius diaperinus* and *Musca domestica*) through partial least squares structural equation modelling, which showed strong reliability (0.6–0.9) and high explanatory capacity (R^2 = 0.6–0.95). Biological potential emerged as the main driver of productive performance, ecosystem contributions showed consistent positive effects, and species-specific challenges reduced predicted outcomes; lower predictive performance for *M. domestica* highlighted limitations of current variables and informed ongoing model refinement. The approach enables comparison of species and management strategies prior to implementation, helping researchers, producers and regulators anticipate trade-offs, prioritise research needs and support evidence-based development of insect-based value chains across diverse agri-food systems, while ongoing work focuses on refining indicators, expanding the species database and improving model performance as new data become available.

GLOBAL PERSPECTIVES AND APPLICATIONS

NutrInsecta: tools for insect bioprospecting, production and nutrition to inform emerging regulation

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NutrInsecta explored the potential of selected insect species for human food and feed to support food security, animal health and evidence-based public policy, using Colombia as a case study relevant to countries developing regulatory frameworks. The project generated decision-support evidence across the value chain, from bioprospecting and prioritisation to production, processing and regulatory dialogue. A database of 50 candidate species was compiled and qualitatively classified by use, productive potential, ecosystem services and challenges. Species patterns were examined using UMAP and geolocation analyses; complementary approaches (e.g., PCA/SEM) were identified as priorities to strengthen prioritisation tools for regulatory applications. Under controlled conditions, growth of *Tenebrio molitor* and *Zophobas atratus* was modelled using nonlinear functions to estimate zootechnical parameters. *T. molitor* frequently followed logistic dynamics, whereas Z. atratus fit Von Bertalanffy trajectories, indicating a slower approach to asymptotic weight but higher biomass accumulation. For processing and characterisation, larvae were analysed at three stages (frozen, paste and meal). Two drying protocols (10 h and 15 h) were compared to optimise efficiency while maintaining nutritional quality. Protein values in *T. molitor* meal were consistent with scientific literature and EFSA assessments. Lipid profiles showed predominance of oleic and linoleic acids in *T. molitor* and stable fatty-acid composition in Z. atratus, with saturated fatty acids of 38.97 ± 0.06% and 36.94 ± 1.03%, and omega-6 between 31.03 ± 0.90% and 33.74 ± 0.77%. To address regulatory gaps, ten interinstitutional working tables were convened, enabling review of 52 ecosystem-service-related species, identification of 14 introduced taxa, development of technical fact sheets and an open-access platform ("insect-hub"), and a production-chain flow map describing 37 products along the value chain. Overall, NutrInsecta integrates prioritisation tools, experimental validation and regulatory articulation to support standardised production, quality-oriented processing and policy development, facilitating formal adoption of insect-derived ingredients in animal feed and, prospectively, human food.

GLOBAL PERSPECTIVES AND APPLICATIONS

Perception of dark chocolate with insect lecithin: impact of information

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The objective of this study was to assess consumer acceptance of dark chocolate formulated with yellow mealworm and black soldier fly larvae lecithin as a sustainable alternative to soy lecithin. Prior formulation optimisation was performed. This assessment was achieved by studying consumer attitudes and responses to labelling and information strategies. Additionally, key sensory attributes such as colour, texture, and aroma compounds were instrumentally assessed. To achieve these objectives, both blind and informed sensory tests were conducted. Blind testing allows for an unbiased evaluation of sensory properties, while comparison with informed testing assesses the psychological impact of transparency and labeling strategies. The results indicated that although there were measurable differences in colour and texture among the formulations, these variations were largely imperceptible to consumers. The aroma compounds of isolated insect lecithin were discriminated using a PCA analysis, however, these differences disappeared when used to formulate dark chocolate. Furthermore, the inclusion of insect lecithin did not negatively impact overall sensory acceptance. However, a trend of preference shifts from insect-based to conventional sources under informed conditions. This suggests that cognitive biases associated with insect-derived ingredients could influence consumer choices. Furthermore, our study confirms that consumer groups with limited or no prior exposure to edible insects exhibit stronger cognitive biases, potentially negatively impacting their acceptance. Addressing this challenge requires effective communication strategies that build familiarity and trust. This underscores the importance of strategic labelling and consumer education in addressing perceptual barriers and supporting market adoption. Transparent disclosure of insect-derived ingredients aligns with ethical marketing principles. This transparency can be further supported by incorporating positive descriptors such as “sustainable” or “eco-friendly” alongside the insect reference, which may enhance initial consumer acceptance without compromising clarity. This research contributes to the advancement of sustainable food systems and offers practical guidance for incorporating innovative ingredients into established food matrices such as dark chocolate.

GLOBAL PERSPECTIVES AND APPLICATIONS

Nutritional profile of the chicatana ant (*Atta mexicana*): a comparison between two regions in the Mexican Southeast

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Chicata ants (*Atta mexicana*) are widely consumed during the rainy season (May–June) in some central and southeastern states of Mexico. This prehispanic tradition serves to ensure food security and provides an income source for the inhabitants of rural and urban areas. Due to its feeding patterns and diverse geographical occurrence, ants are exposed to several abiotic factors, which might confer potential differences in both their nutritional profile and food safety implications. In this work, the analysis of four chicatana ant samples was performed. Two samples were obtained near Tuxtla Gutierrez, Chiapas (530 masl), which is a tropical and hot region, while two samples were selected from Huatusco and Totutla (1300 masl), Veracruz, which are located in a foggy mountainous region with a temperate climate. All the samples were collected, frozen at –18 °C, and freeze-dried for 4 days. To assess possible regional variations in the nutritional profile of such samples, bromatological analysis was performed for the analysis of the moisture (gravimetric method), ashes (furnace method), protein (Micro-Kjeldahl method), and fat (Goldfish method) contents. In terms of bromatological analysis, no significant differences were observed in the fat (35.60-39.40%), protein (35.10–39.40%) and ash (1.50–1.68%) contents. Hence, different abiotic factors did not produce variability in the percentage of different macromolecules. Ongoing research is investigating the regional variations in terms of protein digestibility (IVPD method), microelements (ICP method), along with the content of different antinutrients such as chitin (glucosamine method), phenolic compounds (Folin-Ciocalteu method) and phytates. The latter will determine if different occurrence conditions are related to the nutritional profile of edible ants.

GLOBAL PERSPECTIVES AND APPLICATIONS

Organic waste to clean energy: Briquette du Kivu as a model for valorising urban waste into charcoal in the DR Congo

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Briquette du Kivu is a company operating within the Eastern DR Congo city of Bukavu. To address food insecurity and environmental issues stemming from decades-long regional conflicts, the company produces clean-burning charcoal briquettes for local use as cooking fuel by rural families. This fuel replaces the traditional need to cut down primary growth tropical rainforest to use as wood or wood charcoal, of which 300 ha are lost every year. However, organic food waste cannot be directly carbonized into charcoal and instead must go through an intermediary step to reduce the moisture content and concentrate nutrients. For this, black soldier fly larvae are employed to reduce substrate volume, converting it into frass, which then undergoes pyrolysis in specialized kilns. The output of the kilns is mixed with a clay binder, extruded into cylindrical briquettes, and finally sun-dried to make the final product. Using BSF has the additional advantage of producing larvae, which are used by local farmers to feed livestock. In addition, a percentage of the frass is used directly as fertiliser to grow fruit trees in a nursery operated by BdK. This then provides additional income for rural families to pay for housing, school fees, cell phones, etc. Together, this integrated system addresses several social-environmental and economic issues in an area of ongoing geopolitical conflict.

GLOBAL PERSPECTIVES AND APPLICATIONS

Advances in edible insect research and development: insights from a global symposium in Africa

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Insects are central to human livelihoods because they provide nutritional, environmental, and economic benefits such as food, feed, fertilisers, nutraceuticals and green jobs. Because of these essential benefits, interest in insect-farming innovations, safety concern, public acceptance and marketing opportunities is rapidly growing. Africa faces acute food insecurity and malnutrition and edible insect-farming innovations are a promising nature-based solution. The "Global Symposium on Insects as Food, Feed, and Food Security in Africa", held in Kampala, Uganda from June 5–6, 2025 was organized to understand the challenges and opportunities facing the nascent enterprise in Africa. 109 stakeholders from 12 countries, including researchers, policymakers, nongovernment organizations, farmers, and private sector actors attended. Discussions centred on insects for human food, animal feed, soil health, role of insects in indigenous food systems, commercialization, regulations and experiences from industry. Integration of insects in African farming systems has been accepted. Key barriers are research gaps, inadequate infrastructure, limited funding, and weak regulatory frameworks. There is need to standardize farming technologies, link research to practice, skill-based training, business model development, policy frameworks, scaling proven successes and establishing stakeholder interactive platforms for dialogue. Insect production in African presents a sustainable one health solution that needs effective public-private partnerships and circular economic implementation approaches.

GLOBAL PERSPECTIVES AND APPLICATIONS

BUGS Africa — biomass utilisation by insects for green solutions in Africa

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The Biomass Utilisation by Insects for Green Solutions in Africa (BUGS-Africa) project addresses three challenges in sub-Saharan Africa: organic waste management, climate change mitigation, and sustainable food systems development. Implemented in Uganda, Ethiopia, and Côte d'Ivoire, the project promotes black soldier fly (BSF) bioconversion as a circular, climate-smart technology that transforms organic waste into valuable products such as protein-rich animal feed and organic soil amendments. Throughout 2024 and 2025, a series of country-level workshops brought around 75 government representatives, researchers, and practitioners to exchange knowledge, identify policy and technical gaps, and explore context-specific models for BSF adoption. The workshops also supported dialogue on how BSF technology can be integrated into national waste management systems, agricultural development plans, and climate action frameworks. To assess the feasibility and scalability, BUGS-Africa conducted field assessments, stakeholder consultations, and data collection across the three countries, analysing environmental, economic, and regulatory conditions. Selected sites for assessments were identified as Entebbe and Jinja in Uganda; Addis Ababa and Butajira in Ethiopia; and Yamoussoukro and Dabou in Côte d'Ivoire. These assessments examined legislation and institutional frameworks, the availability and suitability of organic substrates, operational and climatic conditions, and market demand for BSF products including fresh and dried larvae, larvae meal, and frass. Findings highlighted significant organic waste streams with potential for BSF bioconversion. In Addis Ababa, approx. 1.2 million tons of municipal waste are generated annually, about 55% is organic. In Jinja, Uganda, annual waste generation is 122 000 tons, with 43% organic. Across the three countries, findings highlighted the need for clearer regulatory frameworks, targeted capacity building, improved waste management systems, and stronger market development to support BSF scale-up. Recommendations include developing technical standards, investing in pilot and demonstration sites, and strengthening collaboration between government, research institutions, and the private sector. By combining technical assessment, stakeholder engagement, and policy dialogue, BUGS-Africa shows how insect-based bioconversion can be mainstreamed as a scalable solution for sustainable waste management and resilient food systems in Africa.

GLOBAL PERSPECTIVES AND APPLICATIONS

Production characteristics and constraints of black soldier fly farmers in Uganda

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Black soldier fly (BSF) larvae production is one of the fastest-growing insect value chains in Uganda, with potential to lift 4.53 million people over the poverty line and provide annual employment to up to 563 302 people. To contribute to the development of the BSF value chain, this study sought to examine the nature of farmers and management processes as well as constraints faced in BSF production. In a cross-sectional study, BSF farmer-households (n = 83) from three Agro-ecological zones (AEZs): North-Western Savannah grasslands (NWG), Lake Victoria Crescent (LVC) and South-Western rangelands were interviewed using a questionnaire with semi-structured and structured questions. Farmers' main purpose of producing BSF and larvae trait preferred were ranked using Likert scale ranging from 1 (least important) to 5 (most important). Association between demographic and other characteristics of farmers with purpose of production and traits preferred were analysed using chi-square test. In addition, the constraints faced by farmers were ranked. Results Sixty percent (60%) of the farmers interviewed were located in the Lake Victoria crescent. Farmers participating in BSF production were mostly male (68%), slightly over 50% were youth (age 18-35), mostly educated to tertiary level (74%), trained in BSF production (78%) and produced less than 100 kg/week of fresh larvae (75%). Majority of the farmers used the vertical rearing system (68. 67%) compared to the horizontal rearing system (21.69%), sourced starting material from fellow farmers (59%), and raised both flies and larvae (81.9%). Raising BSF to "feed for own livestock" was ranked highest (mean rank = 4.25) followed by income, while the most preferred larvae trait was larvae girth (mean rank = 4.47) followed by length (mean rank = 4.4). The source, availability and importance of substrates used in feeding larvae differed among the AEZs and all farmers purchased substrates off-farm. In all the three AEZs market waste (38.55%), followed by brewer waste (18.06%) were mentioned as most preferred substrates. Inadequate supply of substrate was the most noted constraint (15.6%) followed by death of larvae (12%) and colony collapse (12%). In summary, youth represent a major part of BSF farmers, but BSF farmers are not evenly distributed across the country and are still producing at a low rate primarily because of inadequate supply of substrate. Therefore, to improve BSF production/size of larvae, access to diverse substrates should be prioritized and especially for the youth.

GLOBAL PERSPECTIVES AND APPLICATIONS

From waste to feed: a one health, risk-based roadmap for black soldier fly systems in Saudi Arabia

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With its ambitious Vision 2030, Saudi Arabia is advancing waste management, food security, and the circular economy goals. Yet, much food and other organic waste still ends up in landfills, increasing greenhouse gas (GHG) emissions and wasting valuable biomass. Black soldier fly (BSF) larvae can valorise diverse urban, municipal and agro-industrial organic waste streams into insect meal/oil and frass, with insect-derived ingredients offering a potential alternative to conventional protein sources in animal feeds (e.g., aquaculture), while frass provides an organic soil amendment that can support water retention and nutrient cycling. We conducted a targeted review of local and international literature on food loss and waste, organic side-streams, BSF rearing systems, BSF system LCA studies and risk-assessment approaches for insect-derived feed. This contribution develops a preliminary, risk-based national roadmap for integrating BSF systems into Saudi organic waste management. Indicative outputs (meal, oil and frass) and potential GHG savings, compared with landfilling, were estimated using exploratory scenarios in order to link representative Saudi organic waste streams to potential BSF facilities, which were developed using published parameters (including World Bank datasets). Simultaneously and based on the One Health approach, a qualitative risk-assessment matrix was applied to map exposure pathways and hazards across the value chain (substrate>larvae>processed products>target animals>humans/environment); this approach is guided by national and international sources (e.g., EFSA). According to preliminary scenarios, valorising even a modest fraction of the current organic waste generated could produce notable quantities of locally sourced protein and organic biofertiliser while also reducing pressure on landfills. This supports national waste management plans and also aligns with sustainable solutions. The risk matrix identifies both important controls and the largest data gaps that need to be addressed to safely scale up. These include criteria for substrate eligibility, monitoring for contaminants and pathogens, process hygiene and traceability. This work proposes a phased, risk-based roadmap to support Saudi Arabia's circular, competitive, and productive BSF value chains. In alignment with the OHA and Vision 2030, the roadmap includes pilots with specified substrates, improved monitoring, standards, licensing, digital traceability, and continuous improvement.

GLOBAL PERSPECTIVES AND APPLICATIONS

Toxicokinetics as a framework for contaminant safety in edible insects

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Edible insects are increasingly recognised as key components of sustainable food systems, circular bioeconomy strategies, and alternative protein production. However, their ability to grow on diverse organic substrates raises concerns regarding contaminant transfer, bioaccumulation, and food and feed safety. Current safety assessments of edible insects still rely largely on contaminant concentrations measured at a single exposure time point, providing limited insight into the dynamic behaviour of contaminants within insect tissues. This limits the robustness of risk assessment and regulatory decision-making. Here, we present an integrated toxicokinetic (TK) framework to evaluate contaminant safety across major edible insect species, including *Tenebrio molitor*, *Hermetia illucens* and *Acheta domesticus*. Using harmonised two-phase bioaccumulation assays (uptake on contaminated substrates followed by elimination on clean substrates), insects were exposed to substrates spiked with metals, polycyclic aromatic hydrocarbons (PAHs), and mycotoxins at concentrations aligned with EU feed safety thresholds. Time-resolved sampling, accounting for species-specific life cycles, enabled the application of one-compartment TK models incorporating growth dilution, allowing estimation of uptake and elimination rate constants, bioaccumulation factors, and contaminant half-lives. Results reveal strong species- and contaminant-specific patterns, showing that accumulation is not a uniform trait of "edible insects" as a group but is governed by physiological traits, life history, and contaminant properties. While most contaminants showed rapid elimination and low long-term retention, specific risk-relevant cases emerged, including prolonged Cd retention in *H. illucens*, persistent As in *T. molitor*, and PAH accumulation patterns. These findings demonstrate that safety cannot be inferred from endpoint concentrations alone and must be interpreted in a kinetic context. By shifting from single-point bioaccumulation metrics to toxicokinetics, this work establishes a mechanistic foundation for insect food-and-feed risk assessment. The framework supports evidence-based depuration strategies, species-specific safety thresholds, and substrate management practices, directly informing regulatory science and safe-by-design insect production systems.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Feeding resilience: why Mediterranean food systems need insects

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Mediterranean food systems (MFS) face resilience disruption pressures (climate change, resource scarcity, economic instability, supply-chain breaks) highlighting the need for stabilizing biomass transformation pathways, such as insect production which can convert organic waste into nutrient-rich outputs. However, the role of insect production as resilience stabilizing force in highly endangered MFS remains poorly understood. The study relies on a systematic literature review of experimental and empirical research on insect biotransformation and resilience in MFS. This study examines how insect biotransformation can contribute to food system resilience, focusing on absorptive capacity (AC), adaptability (AD) and recoverability (RC), based on established food system frameworks. Structured searches of major scientific databases targeted peer-reviewed studies on black soldier fly (BSF), yellow mealworm (YM) and other insects used for waste valorisation and nutrient recovery. The literature shows that insect biotransformation strengthens the AC of MFS by valorising waste streams into high-value outputs, reducing greenhouse gas emissions and waste volumes, thereby buffering resource scarcity shocks. Insect production also lowers environmental impacts compared to conventional protein sources and supports closed-loop nutrient flows. Moreover, insect production systems are flexible and adaptable to changing conditions through modularization and biological flexibility. However, research focuses overwhelmingly on AC, while AD and RC remain poorly examined, with limited analysis of system reconfiguration under stress or post-disturbance recovery. Socio-economic and institutional dimensions are largely neglected, economic evidence on farmer cost–benefit trade-offs are scarce, and weak integration with existing food system actors continues reduces the potential for scaling. Insect biotransformation can enhance the AC of MFS through waste valorisation and impact reduction, but its resilience potential remains incomplete. Future research must address AD and RC, alongside technical performance, by developing system-specific resilience indicators, assessing socio-economic feasibility and enabling supportive policy frameworks, as supported by PRIMA program project CIPROMED (No 2231) and project ADVAGROMED (No 101102316).

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Modulating bioactive compounds in black soldier fly larvae through waste feedstocks and industrial processing

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Black soldier fly larvae (BSFL) are increasingly utilised as sustainable bioconversion agents and feed ingredients within circular bioeconomy frameworks. While BSFL biomass is recognised to contain bioactive compounds with antioxidant and immunomodulatory potential, the extent to which rearing diet and downstream industrial processing influence these functional properties remains insufficiently characterised. BSFL neonates were reared under identical commercial conditions on four dietary substrates: a commercial control diet and three waste-derived feedstocks (fruit and vegetable waste, soy okara, and brewer's spent grain). Larvae were harvested at a defined developmental stage, washed, and starved for 24 h prior to freeze-drying. A subset of commercially fed larvae was additionally subjected to an industrial-scale proprietary drying process. Antioxidant capacity was assessed using FRAP, DPPH, and Folin–Ciocalteu assays, while immune-related proteins were characterised using LC–MS/MS proteomics and Gene Ontology-based annotation targeting insect innate immunity. Antioxidant capacity was strongly diet-dependent, with significant treatment effects observed across all assays (DPPH, FRAP, and Folin–Ciocalteu; ANOVA, $p < 0.001$). Vegetable-based diets yielded the highest radical scavenging activity (DPPH), whereas brewer's spent grain consistently enhanced ferric reducing power (FRAP) and total phenolic content. Commercial drying reduced antioxidant activity compared with freeze-dried counterparts. Proteomic analysis revealed parallel diet-driven differences in immune protein composition, driven primarily by immune effector proteins. Vegetable-fed larvae exhibited the highest number of effector-associated proteins (72), followed by soy (61) and brewer's spent grain (56), while core innate immune proteins remained conserved across treatments. Processing exerted a pronounced effect on immune protein retention, with industrial thermal drying significantly reducing the number of detectable immune-related proteins compared with freeze-drying (308 vs 196; $p < 0.05$). Together, these findings demonstrate that both feedstock selection and processing strategy are critical determinants of the bioactive potential of BSFL biomass. By linking antioxidant activity with immune-relevant proteomic profiles, this study highlights opportunities to actively modulate insect-derived bioactive compounds through informed feedstock and processing choices, with implications for sustainable feed, functional ingredients, and insect-derived health products.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Antibacterial activity of *Hermetia illucens* oil against bovine mastitis-associated pathogens

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Bovine mastitis remains one of the most prevalent and economically important diseases in the dairy industry, primarily driven by bacterial infection and exacerbated by the escalating challenge of antimicrobial resistance. This study was designed to investigate the antibacterial efficacy of *Hermetia illucens* (HI) oil against major bacterial pathogens associated with bovine mastitis (namely, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus bovis*, now classified as *Streptococcus gallolyticus*, and *Escherichia coli*), with the aim of identifying a sustainable alternative to conventional antimicrobial therapies. Bacterial growth was monitored over 48 hours in Brain Heart Infusion Broth (BHI) cultures supplemented with increasing concentrations of HI oil (0% as control, 20, 40 and 50%), with bacterial loads quantified as log (CFU)/ml at 0, 24 and 48 hours by plating serial dilutions of each species and concentration. Each species-concentration combination, including the control, was analysed in triplicates at each time point for statistical analyses by means of ANOVA. Growth dynamics were further evaluated using Δ values between 0 and 48 hours to assess treatment-induced variations in bacterial proliferation. The results demonstrated that a 50% inclusion of HI oil significantly inhibited the growth of *S. aureus*, *S. uberis*, *S. agalactiae* and *S. disgalactiae* at both 24 and 48 hours ($P < 0.05$), with mean reductions of approximately 1.4 and 1.2 log units, respectively. Similarly, a significant inhibitory effect against E. *coli* was also observed at 48 hours (–3.2 log units, $P < 0.05$), whereas *S. bovis* showed minimal susceptibility, exhibiting less than 0.5 log reduction with no statistically significant differences among the treatments. This difference may be related to genetic and structural differences between *Streptococcus gallolyticus* (Group D) and the other *Streptococcus* species evaluated. Moreover, the natural habitats of the former are the gut and rumen; therefore, this reduced susceptibility may be related to structural and physiological adaptations associated with its habitat. Such adaptations may include an increase in membrane density and a modified fatty acid composition of the cell membrane, making it less susceptible to destabilization. Overall, these findings highlight the potential of HI oil as a sustainable antibacterial agent for improving udder health and contributing to an antimicrobial resistance mitigation strategy in mastitis management.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

High-yield, time-saving and water-efficient extraction of chitin and chitosan from *Hermetia illucens* breeding waste

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The valorisation of black soldier fly breeding waste offers a promising route for the sustainable production of chitin and chitosan (Elouali *et al*., 2025). However, conventional extraction methods remain resource-intensive and time-consuming. This study evaluates and optimises the extraction of chitin and chitosan from *H. illucens* pupal cases using four chemical processes in order to improve yield while reducing processing time and resource consumption. A conventional extraction protocol was used as a reference (Process 1), while Processes 2 and 3 investigated alternative delipidation strategies using hexane (P2) and a methanol–chloroform mixture (P3). These approaches enhanced lipid removal, reaching 87.87 and 97.27%, respectively. However, only moderate improvements were observed for deproteinization efficiency (21.43% for P2 and 34.58% for P3) and chitin yield (15.43 and 25.72%). To further intensify the extraction process, an autoclave-assisted method (Process 4) was developed and operated at 121 °C and 2.2 bar. Chitin and chitosan obtained from all processes were characterised using FTIR, SEM, XRD, ^{1}H-NMR, TGA, potentiometry, viscosimetry and ICP-OES. Process 4 showed the best performance, with a deproteinization efficiency of 94.25 ± 0.6%, while reducing chemical consumption by 83% and water usage by 80% compared with the conventional process. Reaction time was reduced from 18 h to 75 min with a measured water consumption of 250 ± 26.86 l/kg. This process yielded 34.74 ± 1.15% chitin and 83.33 ± 1.28% chitosan. Physicochemical analyses revealed improved thermal stability, with DTGmax values of 421 °C for chitin and 345 °C for chitosan. Chitin crystallinity decreased by 25.68%, while chitosan exhibited the lowest degree of acetylation and a high molecular weight of 220 378 g/mol. Overall, the autoclave-assisted process provides a rapid and resource-efficient strategy to convert *H. illucens* breeding waste into high-quality chitin and chitosan suitable for sustainable industrial applications. Reference: Elouali, S., Ait Hamdan, Y., Benali, S., Lhomme, P., Gosselin, M., Raquez, J.-M. and Rhazi, M., 2025. Extraction of chitin and chitosan from *Hermetia illucens* breeding waste: a greener approach for industrial application, International Journal of Biological Macromolecules 285: 138302. https://doi.org/10.1016/j.ijbiomac.2024.138302

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Evaluation of the susceptibility of insect meals to infestations by major stored-product beetle species

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The rapid expansion of insect-based meals as sustainable nutrient sources for food and feed raises new challenges regarding storage stability and vulnerability to stored-product insect pests. However, there is a lack of research towards this direction. In this study, we evaluated the susceptibility of three commercially produced insect meals derived from larvae of *Hermetia illucens* (L.) (Diptera: Stratiomyidae), *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) to infestation by nine major stored-product beetle species. Infestation was evaluated as adult survival after 21 days and progeny production after an additional 65 days of incubation. Population growth was quantified using the instantaneous rate of increase. Overall, susceptibility differed among meals and pest species. Both *Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) successfully developed on all insect meals and produced substantial progeny, indicating a high risk of infestation during storage. *Trogoderma granarium* Everts and *Trogoderma variabile* Ballion (Coleoptera: Dermestidae) also developed on all meals, primarily as larval populations, highlighting their potential to persist and contaminate the tested insect-derived products. In contrast, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Leamophloeidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *A. diaperinus* were able to develop on neither of the insect meals, while *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) showed only limited progeny production. These findings demonstrate that several key stored-product beetles can spoil insect meals when stored, underlining the need for targeted storage monitoring and pest-management strategies to protect product quality and prevent post-harvest insect infestations.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Black soldier fly frass: biofertiliser, biostimulant, elicitor or all of the above?

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The frass of the black soldier fly (BSF, *Hermetia illucens*), which is the most important output in volumetric terms of BSF farming, is gaining increasing attention due to its potential to be used for different types of agricultural applications. What makes this product particularly interesting are its high microbial diversity, which includes many microorganisms known to be beneficial to soil and plants, and its chitin content, which might confer it also the ability to elicit plant immunity responses against biotic threats. The research presented here has been conducted in Southern California (United States), in Southern Italy and in the Netherlands, to test the hypothesis that BSF frass can be a fertiliser, a biostimulant of plant growth and an elicitor of plant defences. In Southern California, we have tested a commercial frass as biostimulant and fertiliser of melon plants under greenhouse conditions first and then over three years of field experiments, from 2023 to 2025. We have also tested in the laboratory (in Southern Italy) and in the greenhouse (in the Netherlands) if a frass obtained from a larval diet made of fruits and vegetables (hereafter vegan diet) and a commercial frass, respectively, can be used as biostimulant and defence elicitor of tomato (*Solanum lycopersicum*) and cabbage (*Brassica oleracea*) plants against phloem feeding (the aphids *Macrosiphum euphorbiae* and *Brevicoryne brassicae*) and chewing insects (*Spodoptera littoralis* and *Pieris brassicae*). In Southern Italy we have also tested the efficacy of frass from the vegan diet against two fungal pathogens of tomato (*Alternaria alternata* f. sp. *lycopersici* and *Botrytis cinerea*). The experiments showed that BSF frass: 1. is an effective fertiliser of melon plants, alone and when used to partially replace synthetic fertilisers: 2. negatively affects the development and survival of aphids and caterpillars on tomato plants; 3. can compensate for loss of cabbage biomass caused by caterpillar feeding; 4. can reduce damage by fungal pathogens on tomato. Therefore, our experiments confirmed the versatility of BSF frass as a product that could be used to increase the sustainability of fertilisation and crop protection, although more research is needed to optimise the modes and rates of application and to understand the mechanisms of action.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Effect of fertilization using frass on the phytochemical profile and bioactivity of Italian rapini

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In this study insect (*Tenebrio molitor*, TM) frass and poultry manure has been tested as organic fertiliser in an open field trial for the cultivation of Italian rapini (*Brassica rapa* L. subsp. *sylvestris*). Nine treatments were established in a randomised block experiment with three replications. Treatments consist in 100% conventional mineral fertilisation (control), 100% TM frass, 100% organic fertiliser, 100% poultry manure (PM), 75% mineral + 25% TM frass, 50% mineral + 50% TM frass, 75% mineral + 25% PM, 50% mineral + 50% PM, no fertilisation (negative control). Sample of plants from each plot were harvested from each treatment, pooled and immediately freeze dried and stored. Plant material has been extracted with 80% methanol and the total phenolic content (TPC) and antioxidant capacity, measured as the Trolox equivalent antioxidant capacity (TEAC), ferric-reducing antioxidant power (FRAP) and DPPH· scavenging activity, were determined. The extracts were fully characterised for their phytochemical profile by LC/MS analysis. The application of mixed fertilisers increased the DPPH· scavenging activity of plants compared to individual fertilisation. The TEAC and FRAP ranging from 42.23 to 51.36 μmol Trolox eq/g DM and from 116 to 146 μmol Fe^{2+}/g DM, respectively, did not differ significantly between the treatments. The phytochemicals responsible for antioxidant capacity were phenolic compounds, whose TPC was 6.45–7.72 mg GAE/g DM. Their profile primarily identified derivatives of sinapic, ferulic, p-coumaric, and hydroxybenzoic acids. Flavonoids, including quercetin and kaempferol diglycosides, were less represented, with the highest content found for kaempferol 3-glucosylglucoside. LC/MS analysis revealed that glucosinolates were the dominant metabolites in Italian rapini. Glucoabrassicanapin and glucobrassicin were the most abundant compounds, followed by gluconapin and progoitrin, indicating a prevalence of aliphatic glucosinolates. Insect frass could be used in replacement of chemical fertilisers without affecting antioxidant capacity and metabolite profile of rapini plant. Acknowledgement: Funded by PRIMA Foundation Call 2021, ADVAGROMED project and PAS CNR 2025-2026 bilateral project.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

The influence of frass on the metabolite profile of tomatoes

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The transition to sustainable agriculture has increased interest in alternative organic fertilisers with reduced environmental impact. Frass has emerged as a promising bio-based fertiliser due to its balanced nutrient content, organic matter, and biologically active compounds that can enhance soil fertility, beneficial microbiota, and plant growth and defence. Despite growing interest, the literature devoted to a detailed description of the effect of frass on the plant metabolome is sparse. In this pilot study, we focused on the effect of *Tenebrio molitor*, *Acheta domesticus* and *Grillodes sigillatus* frass on tomato fruits. Frass was applied directly into the soil at a rate of 1 g per 500 ml of substrate at the start of the experiment. First, the evaluation of carotenoid and tocopherol content was performed using high-performance liquid chromatography with a diode array/fluorescence detector. Tomatoes fertilised with *G. sigillatus* frass contained significantly more lycopene, lutein, and α-tocopherol compared to the reference sample and other exposed samples. Next, metabolomic fingerprinting was performed using ultra-high-performance liquid chromatography with high-resolution tandem mass spectrometry with subsequent statistical analysis. Most significant markers that were identified were connected to the stress mechanisms of the plant. Hydroxycinnamic acid amides (HCAAs), compounds contributing to cell wall reinforcement, were, surprisingly, upregulated in the reference group. On the other hand, in frass-fertilised tomatoes, adenine was upregulated. Adenine serves as a precursor of cytokinins, plant hormones regulating growth. Another upregulated metabolite in frass-fertilised samples was stigmasterol, which is a crucial membrane component, influencing cell permeability and signalling. Its levels can change with exposure to stress factors. Monogalactosyldiacylglycerol (MGDG 18:1_18:2) was also increased after fertilisation using frass. MGDGs are part of the chloroplast lipid profile, which changes to maintain cellular homeostasis under stress. Chlorogenic acid was found to be increased in samples fertilised with *T. molitor* frass. Overall, this study represents a valuable insight into the complex metabolic changes related to applying frass as a fertiliser for tomatoes, and can contribute to a better understanding of the potential of using frass in agriculture and to the optimisation of cultivation practices.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

The effects and microbial mechanism of black soldier fly frass application on tomato wilt disease suppression

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Soil organic amendment such as black soldier fly frass (BSFF) has been recently proposed as a promising way to control the occurrence of soil-borne disease. However, the effects and microbial mechanism of BSFF application on tomato wilt disease suppression remains not fully understood. Here we conducted a pot experiment to explore the features of soil microbiome, metabolism and potential contributions to disease suppression after BSFF amending. In total, three treatments with equal nutrient amount input including chemical fertiliser (CF), chicken manure and BSFF were designed in the present study. At the tomato harvest stage, soil samples were collected for further chemical property measuring, pathogen quantifying, 16S rRNA high-throughput sequencing and non-targeted soil metabolomic. Our results showed that compared with the CF treatment, the BSFF treatment improved tomato yield by 7.6-fold, reduced the incidence of tomato fusarium wilt by 87.5%, and simultaneously decreased the soil abundance of *Ralstonia solanacearum* (causing tomato wilt disease) by 96.5%. Concurrently, compared with CF treatment, the BSFF treatment significantly improved soil physicochemical properties, increasing organic matter content by 15.2%, alkali-hydrolysable nitrogen by 27.2% and nitrate nitrogen by 76.1%. Crucially, the BSFF treatment significantly reshaped the tomato rhizosphere microbiome via stimulating a wide range of beneficial taxa strongly linked to disease suppression. Moreover, BSFF application stimulated specific key metabolites associated with plant defence and growth regulation, such as organic acids, flavonoids, and plant hormone precursors as revealed by soil metabolomics analysis. Further correlation analysis indicated that the enrichment of these beneficial bacteria (*Flavisolibacter*,*Lysobacter*, *Hanamia*), along with the accumulation of related metabolites, was several significantly correlated with the reduction in *R. solanacearum* abundance. In conclusion, BSFF could alleviate tomato continuous cropping obstacles through a synergistic mechanism: (1) recruiting beneficial rhizosphere bacterial communities and (2) creating a soil metabolic environment conducive to plant health, thereby potentially activating plant immune responses. This study reveals the role of BSFF in shaping the rhizosphere soil microenvironment and highlights its value as a sustainable soil amendment.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Comparison of black soldier fly frass and mineral fertiliser on phosphorus uptake and maize performance

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Black soldier fly (BSF) rearing is gaining global recognition as a sustainable strategy for valorising organic waste and recycling nutrients, converting biowaste into protein-rich animal feed and nutrient-rich fertiliser. However, the use of BSF frass (BSFF) as an organic fertiliser remains understudied, with limited empirical evidence on its agronomic performance. Previous studies have focused on frass as a nitrogen (N) source, with application rates based on N content. In contrast, this study based application rates on phosphorus (P) content. Accordingly, the study evaluated the comparative effects of BSFF, mineral fertiliser (DAP) and their combined application (DAP + BSFF) on maize growth, P use efficiency, and maize yield. Four P application rates, 0, 50, 100 and 150% of the recommended 30 kg P/acre, were evaluated. BSFF was obtained from Bioconvision, a pilot facility in Uganda, where larvae were reared on 55% water hyacinth, 30% hotel and restaurant food waste, and 15% maize bran. After harvest, the frass underwent 30 days of passive aerated composting and the composted BSFF contained 2.09% N, 2.58% P and 3.26% K (DM basis), 60% organic matter, and had a slightly alkaline pH (7.18). At the 100% P application rate (30 kg P/acre), BSFF increased plant height to 198 cm compared to 86.1 cm in the control, and maize yield improved by 32%. A combined frass and DAP application at 100% P further boosted yield by 44%, outperforming mineral fertiliser alone (38%). Excessive application (150% P rate) led to diminishing returns, with BSFF increasing maize yield by 7.5% and DAP increasing by 6%, underscoring the importance of optimal nutrient application. At optimal rates, BSFF showed slower nutrient release and more stable soil phosphorus concentrations throughout the cropping period compared to DAP, while frass–mineral fertiliser mixture achieved nutrient release and uptake patterns comparable to DAP. Overall, the findings indicate BSFF's potential as an alternative P source and urban organic waste management strategy

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Insect frass as an organic fertiliser and biostimulant in potato production: results from on-farm field trials

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Insect frass from black soldier fly larvae (BSFL) bioconversion contains key components of a fertiliser. It is rich in organic matter and provides a well-balanced spectrum of macro- (N, P, K, S, Mg) and micronutrients (Zn, Mn, Fe, B). In addition, frass is naturally enriched with humic and fulvic acids, chitin and plant-growth-promoting bacteria, all of which are potentially associated with biostimulating effects. Hence, we hypothesized that BSFL frass can at least match the performance of conventional fertilisers in potato production while providing additional benefits for quality and plant health. In collaboration with farmers, BSFL frass was evaluated against locally used conventional fertilisers (mineral or organic fertiliser such as biogas digestate) in eight potato field trials at five locations across Germany. In conventional systems, 2 t/ha of BSFL frass were applied and supplemented with mineral fertiliser to meet crop demand, while in organic systems, the usual fertiliser was fully replaced by 3.5 t/ha frass. Smaller doses (0.5 and 1 t/ha) supplemented with mineral fertiliser were additionally tested to assess potential biostimulant effects. Depending on farm logistics, trials were set-up either as field-long strips (width according to the fertiliser spreader) with four subsamples per strip, or as randomised plot designs with four replicates per treatment. Subsamples of 3.6 m^2 were harvested by hand, and total yield, tuber size distribution, damage by wireworms (*Agriotes*) and fungi (*Rhizoctonia solani*), as well as starch content were recorded. Results differed across sites, but in about 70% of the trials, insect frass matched or outperformed conventional fertilisation. The strongest response was observed on a sandy site, where frass application increased yield by approx. 50%. Effects on wireworm damage depended on pest pressure and weather: under high wireworm abundance, frass did not reduce tuber damage, whereas under lower pressure and site-specific conditions, trends towards reduced infestation were apparent. On sandy soil, frass fertilisation reduced fungal infestation to a similar extent as a standard fungicide treatment. With respect to product quality, starch content tended to be higher under frass fertilisation, although differences were not statistically significant. Overall, current results indicate that BSFL frass is not only a promising organic fertiliser that supplies nutrients effectively but also provides additional functional benefits. Future work should explore this biostimulating potential in more detail, for example with regard to quality improvement and possible enhancement of crop tolerance against drought stress.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Optimised application of insect frass enhances maize performance and soil fertility under alkaline soil conditions

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Insect frass (IF) is a by-product of the insect farming industry that could be valorised as an organic fertiliser in agriculture. However, how IF affects plant performance under alkaline soils is not well understood. This study aimed to assess the comparative effects of *Hermetia illucens* frass, mineral NPK fertilisers and their combination on maize (cv. Dracma) growth, root traits, plant nutrition and soil fertility under greenhouse conditions. Six treatments were applied in a completely randomised bloc design with four replicates as follows: T1 (unfertilised control), T2 (150 kg N/ha using NPK fertiliser), T3 (75 kg N/ha using IF), T4 (150 kg N/ha using 50% IF and 50% NK fertiliser), T5 (150 kg N/ha using IF), T6 (225 kg N/ha using IF). At the V8 growth stage, soil properties, above- and below-ground plant parameters and plant nutrition were assessed. Results showed that soil organic matter increased under T5 and T6 by 18.7 and 16.7% compared to T1 ($p < 0.05$). Similarly, soil available P showed pronounced increases under T6 (171.9%), T5 (130.6%) and T3 (56.2%) relative to T1 ($p < 0.05$). Plant growth followed a similar trend with plant dry biomass increasing under T6, T5 and T3 by 83.5, 68.8, and 61.3% relative to T1 ($p < 0.05$). Root traits recorded the highest increases in T3 with root length, root surface area and root volume increasing by 5.1, 23.6 and 67.4% relative to T1 ($p < 0.05$), respectively. T6, T5 and T3 also outperformed T2 in soil fertility, plant growth and root traits. T6 and T3 showed the highest plant uptake for both macro and micronutrients. Interestingly, T3 increased N use efficiency by 220% while recording almost similar P use efficiency compared to T2. Overall, applying 50% of maize N requirements (75 kg N/ha) using IF optimised maize growth through improvements in soil fertility, root architecture and nutrient use efficiency. Our findings highlight the potential of using IF in Mediterranean agroecosystems for sustainable crop production and nutrient management.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Frass, a high value product or waste?

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The fertilising potential of insect frass has been widely studied, yet reported outcomes remain inconsistent. This study examined whether the feeding substrate of *Hermetia illucens* (BSF) larvae influences the agronomic performance of the resulting frass. Larvae were reared on ten distinct substrates, after which the frass was collected and chemically characterised. Each frass type was subsequently blended with peat to standardize nitrogen (N) input, resulting in mixtures supplying 210 mg N per plant, as determined from a pilot trial. Four replicate pots, each containing four kilograms of the mixture, were sown with 15 Triticum aestivum seeds. Identical controls included a negative control without fertiliser, two organic fertilisers at 210 mg N per plant, and two mineral fertiliser controls supplying either 70 or 210 mg N per plant, reflecting known differences in N availability between manure and mineral sources. Germination was assessed after two weeks, after which plant density was reduced to eight plants per pot. Pots were weighed twice weekly to restore water loss, and evaporation was recorded as a proxy for plant growth. Leaf area was monitored through repeated imaging, and at harvest both above and belowground biomass were measured. Plant and soil composition were analysed to construct a mass balance. A second experiment was performed based on the results of the first. Four frass types (both best and worst) were tested again, but this time with or without the addition of organic soil to improve the microbial degradation and with or without the maturing of frass in the soil for a month as it is a slow-release fertiliser. Despite equal N additions across treatments, fertilisation performance differed markedly: some frass types showed minimal improvement over the negative control, whereas others matched or exceeded the low mineral fertiliser control. These differences did not seem to be correlated with any of the measured chemical parameters. The findings suggest that variability in frass driven by changes in larval substrate may contribute to the inconsistent results reported in the literature. Furthermore this inconsistency in frass does make marketing much more difficult. Future research is needed to find out the cause of this variability and potentially look for other uses such as in perennial plants that can benefit from a slow release fertiliser.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Effect of frass water extracts from four insects and a pre-commercial frass-based formulation on *Meloidogyne incognita*

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Numerous studies have shown that insect frass can enhance soil fertility. However, frass has also recently attracted interest for its plant health–promoting potential. In this study, we evaluated the *in vitro* effects of water extracts of frass from four edible insects, i.e., *Hermetia illucens*, *Tenebrio molitor*, *Alphitobius diaperinus* and *Zophobas morio*, as well as of a pre-commercial frass-based formulation against second-stage juveniles (J2) of the root-knot nematode *Meloidogyne incognita*. Frass batches were divided into two parts: one was directly used for extraction, while the other was heat treated (70 °C for 60 min) prior to extraction. For extract preparation, 1 g of frass was mixed with 15 ml of tap water, followed by sonication for 15 min and filtration through cotton. The bioassays were conducted in 96-well plates, with 25 J2 per well exposed to frass extracts diluted with water to concentrations of 1.5–25% (v/v). Larvae immersed in water served as control. Each treatment had six replicates, and the bioassay was repeated three times. J2 mobility was assessed 24, 48 and 72 h post immersion, after which larvae were classified as mobile or paralysed. After the final assessment, J2 were transferred to water; individuals that did not regain motility were considered dead. The same procedure was followed for the pre-commercial formulation. A clear dose response relationship was established in all cases. In some cases, a nematostatic effect was evident at 48 h, but paralysis depicted after 72 h was in all cases irreversible. Most importantly, non-heat-treated frass extracts exhibited higher nematicidal potential and the EC_{50} value was calculated at around 10% (v/v) for all four insects. A dose dependent paralysis was evidenced also for the pre-commercial product, causing 70% mortality above control levels at 1.5% (v/v). To conclude, the results demonstrate the nematicidal potential of frass extracts of *H. illucens*, *T. molitor*, *A. diaperinus* and *Z. morio* against *M. incognita* larvae. Additionally, for the first time, the nematicidal activity of a pre-commercial frass-based formulation against *M. incognita* J2 is reported. Further experimentation is warranted to investigate the *in vivo* effects of frass extracts against root-knot nematodes.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Black soldier fly frass as fertiliser: evaluation on nutritional effects in the vineyard over a period of two years

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Black soldier fly larvae frass (BSFF) contains macro- and micronutrients, humic substances, chitin and plant-growth-promoting microbes, highlighting its potential as both fertiliser and biostimulant. This study evaluated BSFF as an organic fertiliser on grape yield and quality over two growing seasons (2024–2025) under variable climatic conditions. The experimental vineyard included 2200 Nebbiolo vines grafted onto Kober 55BB. Each spring, BSFF was manually applied along the rows and incorporated into the soil mechanically. Based on soil and frass analyses, two rates were tested: 1400 kg/ha (A) and 2100 kg/ha (B). A manure-based fertiliser served as control. To verify whether plant responses were independent of larval diet, two BSFF types were used: vegetable-based (NPK 2.6-1.6-1, 2024) and wheat bran-based (NPK 3-4-3, 2025). Across both years, 660 vines were included in treated rows (220 per row). For measurements, 20 vines per treatment were labelled and monitored from spring to harvest. Recorded parameters included shoot length, number of inflorescences, bunch weight and must composition (pH, acidity, sugars and yeast assimilable nitrogen, YAN). Data were collected in triplicate and analysed using R. In both seasons, BSFF significantly increased bunch weight compared to the control. In 2024, mean bunch weight was 307.8 ± 86.38 g (A) and 282.4 ± 125.01 g (B), versus 170.4 ± 65.46 g in the control. In 2025, values were 313.0 ± 135.17 g (A) and 282.1 ± 132.65 g (B), compared to 175.2 ± 58.65 g in the control. No significant differences were found between A and B rates. YAN, a key indicator of nitrogen availability for yeast and fermentation performance, was also higher with BSFF. In 2024, YAN reached 180.0 ± 8.00 mg/l (A) and 146.0 ± 4.00 mg/l (B), compared to 85.7 ± 7.51 mg/l in the control, with significant differences between A and B ($p = 0.002$). In 2025, YAN values were 34.0 ± 2.65 mg/l (A) and 34.0 ± 7.21 mg/l (B), versus 20.0 ± 21 mg/l in the control, with no difference between rates ($p = 0.997$). Overall, BSFF proved effective as an organic fertiliser for vines, increasing bunch weight and improving YAN regardless of larval diet or annual climatic variability. Further research will investigate its biostimulant effects and impact on soil quality.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Valorisation of frass and exuviae from *Hermetia illucens* as a tool for reducing *Fusarium* wilt disease on lettuce

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The increasing global demand for sustainable food and feed requires the development of circular production systems aimed at reducing agri-food waste and providing alternative nutrient sources. Insect farming represents a promising solution, converting organic by-products into valuable protein and generating insect frass, composed of excrements, exuviae and residual substrates. Several studies have reported the ability of insect frass to control Fusarium spp. *in vitro*, due to its content of chitin, chitosan and beneficial microorganisms. *Fusarium oxysporum* f. sp. *lactucae* is a soil-borne pathogen causing severe wilt disease in lettuce. The aim of this study was to evaluate the capacity of frass and exuviae from *Hermetia illucens* to suppress *Fusarium* wilt on lettuce under greenhouse conditions. Frass and exuviae were obtained from *H. illucens* larvae reared on Gainesville diet or agri-food by-products. Three greenhouse trials were conducted on potted lettuce plants. Heat-treated frass from Gainesville diet (70 °C, 60 min) was mixed with peat at 10% (v/v), while frass from food by-products was applied at 1, 5 and 10% (v/v), and insect exuviae at 1% (v/v) at sowing. Seven days before transplanting, substrates were inoculated with *F. oxysporum* f. sp. *lactucae* race 4 (1 g/l wheat kernel biomass). Untreated inoculated controls and a chemical control (azoxystrobin, 1 ml/l) were included. Disease incidence, disease severity (0–4 scale) and above-ground biomass were assessed. Data were analysed by ANOVA followed by Welch Games-Howell test. In all trials, *Fusarium* wilt incidence and severity were significantly reduced by heat-treated Gainesville frass at 10% and food by-products frass applied at 5% and 10%. *Fusarium* wilt incidence was also significantly reduced by food by-products frass at 1%, while 1% exuviae was not effective. Gainesville frass at 10% showed comparable efficacy than the fungicide treatment. The results highlight the potential of *H. illucens* frass as a sustainable soil amendment for *Fusarium* wilt management in lettuce. Frass applications significantly reduced disease severity, probably thanks to the content in chitin, which is known to induce plant resistance and suppress soil-borne pathogens, and the native microbiota. Although further trials are required, insect frass could partially substitute fertilisers and fungicides in protected and open-field lettuce production. Acknowledgement: This research was conducted within the PNRR E.U. – NextGenerationEU initiative under the National Research Centre for Agricultural Technologies – AGRITECH (CN00000022).

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Insect frass-based fertilisation as an agroecological strategy for Mediterranean tomato cultivation

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In recent years, insect frass, defined as a mixture of insect faeces, shed exoskeletons, and residual substrate of reared insects, has garnered significant interest for its potential applications in agriculture and its environmental sustainability. It has been estimated that this by-product could represent a substantial proportion of the production system, typically around 50-75%, of the total biomass produced in insect farming. A two-year open-field agronomic trial was carried out in Mola di Bari (BA, Apulia, Italy) on tomato (*Solanum lycopersicum* L.) cv. "Regina" (1st trial: May–August 2023; 2nd trial: April–July 2024). Nine treatments were arranged in a randomised complete block design with three replicates: (1) chemical fertiliser; (2) *Tenebrio molitor* frass; (3) organic fertiliser; (4) poultry manure; (5) chemical fertiliser + frass; (6) chemical fertiliser + organic manure; (7) chemical fertiliser + poultry manure; (8) chemical fertiliser + poultry manure; and (9) unfertilised control. Fertilisers were manually applied before transplanting. For each treatment, fertiliser rates were calculated to supply 130 kg N ha^{-1}. During the growing cycle, bio-morphometric parameters, yield, fruit quality, and total microbial load in the rhizosphere were assessed. Metagenomic metabarcoding analysis was performed for each treatment on rhizosphere samples collected at a depth of 30 cm to evaluate changes in the microbial community during the cultivation period. Plants fertilised with insect frass showed yields comparable to those obtained with chemical fertilisers. Similarly, the qualitative and mineral profiles of the fruits were comparable among treatments. Metagenomic analysis revealed shifts in rhizosphere microbial community composition in terms of abundance and diversity, with significant differences observed in chemical fertiliser treatment compared with *Tenebrio* frass and unfertilised control. These findings highlight *T. molitor* frass as a viable, sustainable alternative to synthetic fertilisers, capable of maintaining crop yields and fruit quality. Furthermore, the observed modulation of the rhizosphere microbiome suggests that frass application may promote soil biodiversity and long-term health, supporting the transition toward circular agricultural systems The present work has been funded by the PRIMA Foundation Call 2021 in the framework of the ADVAGROMED project.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

The potential of enteric methane mitigation in cows by frass from black soldier fly larvae bioconversion

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Frass from black soldier fly larvae (BSFL) bioconversion of organic residual streams is a potent fertiliser. Beyond this, we assessed whether due to its nutrient composition, microbial profile and chitin content, insect frass can be a ruminant feed and reduces enteric methane production. This is because chitin and lactic acid bacteria, originating from larval exuviae and the bioconversion process have both been previously associated with reduced methane (CH_4) formation in the rumen. In this study 5 different BSFL frass types were examined: non-hygienised frass of different feed substrates (Frass 1 and Frass 2), frass mixed with exuviae at two inclusion rate, and hygienised frass (heat treated at 70 °C for 1 h). Firstly, the study determined inclusion rates of these BSFL frass types based on nutritional analyses (crude protein, lignin, fibre and ash content). Then the *in vitro* digestibility and the Hohenheim Gas Test (HGT) was applied to quantify CH_4 formation (ml CH_4 per ml/g, per *in vitro* organic matter degraded (IVOMD) and per total volatile fatty acids (VFA)). Nutrient analysis revealed that the tested frass types had similar composition than high-value ruminant feeds. Hence, a replacement of 20% of alfalfa hay into a basal diet on a dry matter (DM) basis was selected for the frass types to perform the HGT. There the basal control diet consisted of 700 g/kg DM hay and 300 g/kg DM concentrated meal. A good feed value of some BSFL frass types for ruminants was confirmed *in vitro* showing similar or only slightly lower digestibility values (52-57%) relative to the control (60%). Frass derived from two different BSFL diets significantly reduced absolute CH_4 production by 27% (Frass 1: 26 ± 2 ml/g CH_4) and 39% (Frass 2: 22 ± 2 ml/g CH_4), respectively, compared with the control diet (36 ± 2 ml/g CH_4). Normalized to 100% IVOMD or to total VFA, the CH_4 yield for the insect frass was 15-23% lower for Frass 1 and 2 compared to the control, demonstrating a genuine anti-methanogenic effect. Interestingly, hygienisation of BSFL frass (32 ± 2 ml/g CH_4) and the inclusion of exuviae based chitin (30 ± 2 ml/g CH_4 and 31 ± 2 ml/g CH_4, respectively) attenuated the anti-methanogenic effect relative to non-treated frass (26 ± 2 ml/g CH_4), suggesting that processing conditions and structural components critically influence the functional properties of frass in the rumen. Overall, the results indicate that BSFL frass can serve as a potential ruminant feed supplement with positive effects to mitigate enteric CH_4 emissions, while highlighting the need to balance methane reduction with maintenance of digestibility.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Valorising insect by products as natural antifungal agents for postharvest protection

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This study investigates the potential of insect-derived bioactive components to reduce storage losses in agricultural value chains, with a particular focus on suppressing *Fusarium* growth in potatoes. As part of the INTERREG DODILog project, unavoidable waste streams from black soldier fly (*Hermetia illucens*), i.e. pupal cases and post-reproductional flies, and mealworm (*Tenebrio molitor*), i.e. post-reproductional beetles, were characterised and evaluated for their natural antifungal properties (i.e. for bioactive molecules like chitin, antifungal peptides and antioxidants). Analytical results showed strong compositional differences: chitin content ranged from 15.6–18.5 g/100 g in dry fly samples, 21.2–21.6 g/100 g in mealworm beetles, and peaked at 23.7–25.6 g/100 g in BSF pupal shells. Protein levels were comparable across sources, with means of 42.6% for flies, 41.3% for beetles and 39.3% for pupal shells. Antioxidant capacity was highest in shells, reaching 176.7 mg/100 g (DPPH) and 452.6 mg $FeSO_4$/100 g (FRAP), compared to 59.0 mg/100 g and 202.3 mg $FeSO_4$/100 g in flies, and only 26.1 mg/100 g and 44.1 mg $FeSO_4$/100 g in beetles. To enhance release of peptides and chitin, insect biomass was fermented, for each sample separately, with *Bacillus subtilis*, producing proteases, organic acids and antimicrobial metabolites. Fermented extracts and isolated peptides, coming from the 3 different samples separately, were tested using agar-plate droplet assays against *Fusarium*. Both extracts and peptides showed visible inhibition zones, with undiluted extracts consistently outperforming 10× dilutions. Isolated peptides produced lighter but clear inhibition. Application tests on *Fusarium*-infected potato slices further demonstrated reduced fungal development on the treated parts compared with the untreated control, indicating the practical antifungal potential of the extracts. Overall, insect residues — particularly pupal shells due to their approx. 25 g/100 g chitin and high antioxidant capacity — represent a promising, circular source of natural antifungal compounds. This research indicates that fermented extracts and peptides might be able to suppress Fusarium, with the strongest effects observed in undiluted fractions. Future work will optimise extraction, concentration requirements for spray applications, and scalability within agricultural storage systems.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Wool dyeing with protein hydrolysate from black soldier fly

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Synthetic dyes are among the most widely used chemical compounds across various industrial sectors, including textiles. However, their high toxicity and the presence of non-biodegradable components raise significant environmental issues with their use. This scenario has spurred a growing search for sustainable, environmentally friendly alternatives, prompting renewed interest in natural dyes derived from plants, microorganisms, insects, minerals, and molluscs. In this study, proteins and pigments extracted from black soldier fly (BSF) were used as natural dyes for wool fabric dyeing. BSF larvae, cocoons, and flies fed with waste from large-scale retail trade were previously chopped and defatted with hexane. The extraction of the proteins was performed in a reactor under pressure at 170 °C for 1 h, using only water as the solvent. The extracted proteins were used to dye wool fabrics with and without mordant (ferrous sulphate). Dyeing was performed with an Ahiba Nuance Top Speed II laboratory dyeing apparatus at a temperature of 90 °C for 1 h, on wool fabrics treated with concentrations variables of protein hydrolysate used as a dye from 2% to 50% by weight of the fibre (o.w.f.) at a pH of 4.5 and bath ratio 1/40. Visually, the woollen fabrics, which were initially white/cream, were dyed in various shades of brown. The effectiveness of the treatment was evaluated by measuring the colour strength (K/S value) of the dyed fabrics. It is observed that fibres treated with hydrolysate protein derived from BSF larvae, without a mordant, show an increase in K/S from 0.32 to 1.44 as the dye concentration increases from 2% to 50%. Fabrics dyed with hydrolysates from cocoons and flies show a similar trend. Still, their K/S value is higher at all concentrations compared to the K/S value of wool dyed with dye extracted from larvae. High K/S values with mordant may be due to dye anchoring or ferrous sulphate, which darkens the shades. Overall, the present work highlights the potential of protein hydrolysate from BSF as a natural, eco-friendly dye, which may represent a promising alternative to synthetic dyes in the textile sector. Acknowledgements: The authors thank for his financial support of the project HI-Tech- PRIN 2022 — D.D. MUR n.104 02/02/2022, Italy, and Prof. Laura Gasco for BSF materials.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Natural Deep Eutectic Solvents as green way to extract protein, chitin and obtain chitosan from *Tenebrio molitor* beetle

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Beetles of *Tenebrio molitor* is a by-product of mealworms production that cannot be used for food and feeding purposes since the strong bond between protein and chitin hinders digestibility. Natural Deep Eutectic Solvents (NADES) are green solvents that can replace conventional and harmful extraction methods currently used to obtain protein and chitin. In addition, NADES can convert chitin into chitosan through deacetylation, obtaining chitosan with many industrial applications due to higher solubility and bioactive properties. In this study, different NADES were tested to extract protein and chitin from *T. molitor* beetles in a single step. The effects of time, temperature, and molar ratio were studied to optimize the extraction process. After, the chitin was converted into chitosan developing a novel method using NADES. For that, distinct NADES, duration of treatment and temperatures were evaluated, assessing the degree of deacetylation (DD) of the resulting chitosan using FTIR, with low (until 30%) or high (>50%) DD indicating chitin- or chitosan-like compounds, respectively. The most suitable NADES to extract protein and chitin from beetles was formed by betaine and urea (Bet:Ur). The optimization of protein and chitin extraction using the Bet:Ur NADES (1:2 molar ratio, 30% water, 90 °C, 3 h) maximized protein hydrolysis up to 81.5%, enabling a highly efficient separation of both fractions. After, varied NADES were tested to deacetyl the chitin into chitosan, with the FTIR analysis showing higher DD using the NADES formed with betaine and glycerol (38.1%) and Bet:Ur (34.6%). Since low DD was achieved using solely NADES, an additional step using an alkaline solution was performed after NADES. Varied concentrations of NaOH were screened (up to 20%, w/v). As a result, using NADES formed with Bet:Ur and NaOH 20% w/v increased the DD of the resulting chitosan, such as 61.1% and 52.3% when commercial chitin or from *T. molitor* beetles chitin was treated in these conditions, respectively. The NADES composed of betaine and urea efficiently pretreated chitin, exposing the acetyl groups and allowing using a low concentrated alkaline solution to replace the acetyl by amine groups. With this approach, highly deacetylated chitosan was obtained, developing an alternative and eco-friendly method that may replace the chemical procedures currently used. Also, since high DDs of chitosan is directly associated with high solubility, the chitosan herein obtained may have varied applications in distinct fields.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Fermentation-driven enhancement of functional and sensory properties of edible insects for sustainable food applications

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Edible insects are high-quality protein sources, yet their food application is hindered by characteristic off-flavours and a lack of processing strategies that simultaneously improve functional and sensory qualities. This study proposes fermentation as a platform technology to overcome these barriers. To evaluate functional enhancement, lactic acid fermentation was applied to plant-based systems (okara and soymilk) supplemented with insects. Okara was selected as an excellent fermentation substrate for edible insects, providing essential fermentable carbohydrates for *Lactobacillus brevis*, while a 30% insect ratio was established to optimise microbial growth without overwhelming the sensory profile. Fermented okara with 30% *Tenebrio molitor* or *Protaetia brevitarsis* larvae showed a marked increase in γ-aminobutyric acid (GABA), reaching 399 and 256 mg/100 g, respectively, compared with 173 mg/100 g in the okara control. Similarly, soymilk fermented with pressed *T. molitor* larvae exhibited significantly higher GABA (122.52 μg/ml) than the control (21.58 μg/ml). To modulate sensory profiles and develop insect-based seasonings, traditional Korean Sikhae (fermented grain) fermentation methods were independently applied to insects. Flavour volatiles of the Sikhae-type products were analysed via gas chromatography-mass spectrometry (GC-MS). In these products, millet supplementation promoted fatty and smooth flavour notes; the relative peak area of methyl tetradecanoate in ground *T. molitor* with millet reached 772.47×10^2, versus 154.50×10^2 in the millet-free control. Furthermore, high-functional Meju (fermented soybean bricks) was prepared with soybeans, okara, and *T. molitor* (4:3:3 ratio) and fermented with *Aspergillus oryzae* to produce low-salt soy sauce. Parallelly, fermented soybean paste-based seasonings using *T. molitor*, *P. brevitarsis* and *Gryllus bimaculatus* were prepared. Electronic nose analysis revealed these processes generated roasted, cocoa-like, and umami-rich aromas driven by pyrazines and ketones, with the *T. molitor* seasoning achieving the highest amino-type nitrogen content. For sensory evaluation, consumer tests (n = 100, ages 10s–60s) were conducted in the US, where the seasonings were applied to a roasted broccoli dish. The results showed the *T. molitor* seasoning attained the highest overall preference score (6.85/9), while the *P. brevitarsis* seasoning exhibited superior *in vitro* antioxidant and anti-diabetic activities. Overall, these fermentation-driven strategies provide an effective foundation for transforming edible insects into high-value functional ingredients and foods with improved consumer acceptance.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Cricket-derived chitosan as a sustainable clarifying agent for apple juice

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Edible insects, particularly the house cricket (*Acheta domesticus*), are increasingly recognized as sustainable resources whose processing generates chitin-rich residues that can be valorised into chitosan, a biodegradable multifunctional biopolymer. This study evaluated the potential of cricket-derived chitosan as a natural clarifying agent for apple juice using cloudy commercial apple juice as a model system. Chitin was extracted from crickets and converted to chitosan, yielding 7.01% chitin and 3.85% chitosan. The degree of deacetylation (DD) was determined by FT-IR spectroscopy as 81.07% for cricket-derived chitosan and 77.36% for the commercial shrimp-shell-derived chitosan as reference. Clarification experiments were conducted using cricket-derived chitosan, commercial chitosan, and a control sample. The control consisted of apple juice subjected to identical mixing and centrifugation without any clarifying agent, representing turbidity reduction due solely to centrifugation. Juice quality was evaluated by turbidity, total phenolic content, antioxidant activity (ABTS), pH, °Brix, and colour measurements. Cricket-derived chitosan achieved near-complete clarification of apple juice, reducing turbidity from 1066.67 ± 31.26 NTU to 2.90 ± 0.10 NTU, outperforming both the control (712.10 ± 6.42 NTU) and commercial chitosan treatment (189.23 ± 1.10 NTU), with significant differences among all groups ($p < 0.05$). These results highlight the strong adsorption capacity of insect-derived chitosan toward haze-forming colloids. Compared to the control, phenolic content decreased by 21.3 and 48.9% following treatment with commercial and cricket chitosan, respectively, while antioxidant capacity decreased by 4.46 and 17.5%. These changes are mainly attributed to electrostatic interactions between protonated amino groups and phenolics, together with hydrophobic and π–π interactions enhancing adsorption efficiency. Despite partial reductions in bioactive compounds, the observed losses remained within acceptable limits considering the substantial improvement in clarity and stability. Overall, this study demonstrates the strong potential of cricket-derived chitosan as a sustainable alternative to conventional clarifying agents, enabling the valorisation of insect processing by-products within a circular economy framework. Future studies integrating life cycle assessment and techno-economic analysis are required to assess its industrial feasibility and environmental performance in large-scale food processing systems.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Tenebrio molitor proteins concentrate as a natural emulsifier in oleogel-based HIPEs

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Oil structuring has gained popularity as a possible alternative for developing new products with a better nutritional profile, particularly those that are trans fat-free, low in saturated fatty acids, and high in polyunsaturated fatty acids. High internal phase emulsions (HIPEs) containing gelled oil phases have shown great potential due to their stability, adjustable shape and microstructure, and promising mechanical and functional qualities. In this context, proteins have emerged as excellent natural emulsifiers, offering a more sustainable alternative to inorganic particles and synthetic surfactants. Insect proteins, as protein-based emulsifiers, have lately garnered more interest due to their reduced environmental effect relative to dairy proteins, aligning with consumer desire for more sustainable protein sources. This study examined oleogel-in-water emulsions (75:25, w/w) formulated with whey protein isolate (WPI) and *Tenebrio molitor* proteins concentrate (TMPC) at concentrations of 4% and 2.5% in water. To produce oleogel, beeswax (BW) at 2% (w/w) and rice bran wax (RBW) at 1% (w/w) were investigated in the sunflower oil phase. Emulsions were characterised by optical microscope, oil loss, particle size and distribution, zeta potential and rheological measurements. The TMPC-stabilized emulsions showed droplet sizes ranging from 20.36 to 25.12 μm, whereas the WPI-stabilized emulsion had larger dimensions (22.93–36.22 μm). The zeta potential values for all four emulsions ranged from –31.8 to –37.8 mV, with the formulation containing 2.5% TMPC and 1% RBW exhibiting the greatest negative value. Furthermore, oil loss reflects this trend by indicating a higher oil loss percentage in samples processed with WPI rather than TMCP. Due to the decreased stability of WPI-stabilized emulsions, only TMPC-based emulsions have undergone rheological testing. Both emulsions displayed a linear regime at low strain levels, with the storage modulus (G') being greater than the loss modulus (G''), confirming the gel-like features of the emulsions. All structures showed a reduction in viscosity as shear rate increased, indicating shear-thinning behavior typical of pseudoplastic fluids. These findings revealed that TMPC-stabilized HIPEs with gelled oil phase outperformed WPI-based formulations in terms of self-supporting qualities.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Integrating insect protein into sustainable food systems: a TEA of *Tenebrio molitor*-enriched bread

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Insect-based foods are increasingly viewed as efficient protein sources that can support sustainability transitions in agri-food systems. However, evidence on their industrial-scale economic performance and sensitivity to policy incentives remains limited. This study conducts a techno-economic assessment (TEA) of bread enriched with *Tenebrio molitor* within the InsectERA Mobilising Agenda, focusing on cost structures, scale effects, and implications for policy-driven market uptake. The analysis integrates production and cost data with process-based modelling of insect-derived ingredients, encompassing rearing on wheat bran, processing steps, and outputs such as ground larvae and frass, alongside downstream bread manufacturing. Mass and cost balance models estimate CAPEX, OPEX, unit production costs, and payback periods under alternative production and market scenarios. The framework reflects an industrial bakery context, accounting for inputs, labour, energy use, processing requirements and packaging. Scenario and sensitivity analyses examine how economic outcomes change with production scale, input prices, and potential cost-reducing measures. Findings show that *Tenebrio molitor*-enriched bread is compatible with standard baking processes, while economic performance depends heavily on insect ingredient prices, production volume, and operational efficiency. OPEX is driven mainly by raw materials and processing costs, whereas CAPEX is linked to baking and preparation equipment. Scenario analyses reveal that scaling up production and moderately reducing insect ingredient costs substantially improve unit costs and payback periods. These results highlight opportunities for targeted policy instruments – such as investment grants, innovation support, and transitional incentives – to strengthen early market development. By applying a TEA approach to a commercial insect-based food, the study clarifies the economic thresholds for market viability and shows how policy interventions may accelerate protein diversification and support broader integration of insect-based foods into mainstream food systems.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Black soldier fly larvae meal *in vitro* assessment: antioxidant capacity, cytotoxicity, and impact on epithelial barrier

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Black soldier fly larvae meal (BSFLM) is a promising protein source for monogastric animal nutrition, with potential gut health benefits. Data on BSFLM bioactivity after modified static simulated gastrointestinal digestion (MSSGID) and intestinal epithelia interactions are scarce. This study evaluated antioxidant capacity, cytotoxicity, and epithelial barrier integrity of digested BSFLM *in vitro* using Caco-2 (human colorectal adenocarcinoma) and IPEC-J2 (porcine jejunal epithelial) cell lines. Defatted BSFLM (49.51% crude protein) underwent MSSGID *in vitro*. Antioxidant capacity was assessed in undigested BSFLM via ABTS assay and in digested BSFLM via DPPH assay at 30-, 60-, and 90-min. Both cell lines were exposed to digested BSFLM for 4 h. Cell viability was quantified by MTT assay. Barrier integrity was evaluated by TEER in monolayers on Transwell inserts, confirming tight junction preservation. Digested BSFLM radical scavenging showed dose- and time-dependent increase, with values from 2.1% (30 min) to 9.8% (90 min) for 100 μg/ml, and from 18.6% (30 min) to 41.39% (90 min) for 800 μg/ml. Cell viability was optimal (>85% vs controls) at ≤400 μg/ml in both cell lines, but higher doses were cytotoxic. TEER values at 400 μg/ml showed no significant changes (residual% of baseline: Caco-2, 106.1 ± 7.9, $p = 0.10$; IPEC-J2, 76.6 ± 3.6, $p = 0.99$), indicating preserved epithelial integrity and barrier function. Our data suggest that BSFLM retains antioxidant properties post-digestion, low cytotoxicity, and no compromission of intestinal barrier. Time-dependent radical scavenging enhancement may reflect bioactive peptides/compounds release during incubation. Preliminary results support BSFLM incorporation in monogastrics' diets to promote intestinal health. Ongoing investigations on immune-modulatory effects, barrier integrity markers, and metabolomic profiling of bioavailable compounds will further elucidate BSFLM's mechanisms. Acknowledgement: The authors thank nextProtein for supplying the black soldier fly meal.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Insect meals in Nile tilapia diets: performance, digestibility and response to *Streptococcus agalactiae* challenge

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Nile tilapia (*Oreochromis niloticus*) is a major freshwater aquaculture species, and insect meals are promising alternatives to conventional protein sources in aquafeeds. This study evaluated the effects of partially replacing an insect-free control diet with larval meals from *Tenebrio molitor* and *Hermetia illucens* on growth performance, nutrient digestibility, haematological profiles and resistance to *Streptococcus agalactiae* in juvenile Nile tilapia. Fish were fed isonitrogenous and isoenergetic diets for 82 days: a control diet without insect meals (CON) or diets containing 100 g kg^{-1} (dry matter) of *T. molitor* meal (TM) or *H. illucens* meal (HI). Growth performance did not differ among treatments ($P > 0.05$), while feed conversion ratio showed a near-significant dietary effect ($P = 0.0525$), with HI exhibiting numerically poorer feed efficiency than TM. Somatic indices were not affected ($P > 0.05$), although liposomatic index showed a near-significant response ($P = 0.0544$). Apparent digestibility coefficients indicated consistently high lipid digestibility and moderate-to-high protein digestibility; dietary and ingredient protein digestibility differed among insect sources ($P < 0.05$), whereas lipid digestibility did not. Erythrocyte and total leukocyte counts were unchanged, but insect-fed groups showed lower neutrophil and higher lymphocyte proportions than CON ($P < 0.05$), suggesting mild modulation of basal leukocyte distribution. After intraperitoneal challenge with S. agalactiae, cumulative mortality was numerically lower in TM and HI than in CON, although Kaplan–Meier survival curves did not differ among treatments (log-rank test, $P = 0.592$). Overall, *T. molitor* and *H. illucens* meals can be included at 10% in juvenile Nile tilapia diets without impairing performance, supporting efficient nutrient utilisation and modest shifts in leukocyte profiles, with descriptive indications of improved outcomes under bacterial challenge.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Effect of BSFL frass tea and UV light exposure on growth, nutritional quality and microbiome of beet microgreens

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Microgreens are valued for rapid production and high nutrient density. Black soldier fly larvae (BSFL) frass tea, an aerated aqueous extract of frass standardized to 0.9 g N/l and supplemented with 0.5% molasses, is a nutrient- and microbe-rich biofertiliser that may reduce reliance on synthetic fertilisers. Ultraviolet (UV) radiation can further modulate plant growth and phytochemical accumulation, yet its interaction with BSFL frass tea in betalain-rich beet microgreens remains unclear. This study examined the effects of BSFL frass tea and UV exposure on growth, nutrient composition, antioxidant activity, and microbial dynamics of beet microgreens. Microgreens were grown for seven days in a factorial design crossing greenhouse film type (UV-transmitting vs. UV-blocking) with three nutrient treatments (water, nutrient solution, or BSFL frass tea). Stem elongation was recorded daily. Leaf area and shoot and root biomass were measured at harvest. Dry matter, nitrogen, ash, betalains, chlorophyll and antioxidant activities were quantified. Microbial abundance and community composition were characterised in BSFL frass, frass tea, growing media, and harvested microgreens using 16S rRNA amplicon sequencing to assess beneficial and pathogenic taxa. Data were analysed by ANOVA with Tukey's HSD ($\alpha = 0.05$). BSFL frass tea increased leaf area and total biomass by 19–21% relative to water-grown controls but remained 28–35% lower than nutrient solution treatments. UV exposure did not affect biomass but strongly altered pigment and antioxidant profiles. Under UV-blocking conditions, nutrient solution produced the highest betacyanin content; however, under UV exposure, frass tea and water increased betacyanin levels by 81–153% relative to nutrient solution. Frass tea enhanced plant antioxidant activities by 56–65% under UV exposure compared to nutrition solution, indicating improved stress resilience. Microbiome analysis showed increased bacterial load from frass to frass tea without changes in dominant taxa (*Atopostipes* sp. and *Vagococcus* sp.) or alpha diversity. Although frass-derived taxa did not dominate the growing media, frass tea increased beneficial bacteria, including *Azospirillum* spp. and *Pseudomonas* spp. No notable foodborne pathogens were detected on harvested microgreens. Overall, BSFL frass tea moderately enhanced microgreen growth, increased UV-induced antioxidant responses, and promoted beneficial microorganisms without introducing foodborne pathogens, supporting its potential as a safe, sustainable biostimulant for microgreen production.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

The effect of insect frass application on nutritionally valuable minerals in radish

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Frass is defined as a by-product of insect rearing, consisting of residual substrate that includes spent feedstock, insect excreta, and cuticular remains. Due to its beneficial chemical composition and agronomic properties, it has been evaluated as a potential biofertiliser comparable to other organic fertilisers. The aim of this study was to assess the effect of frass derived from the rearing of yellow mealworm larvae (*Tenebrio molitor*) on the content of nutritionally valuable mineral elements in radish (*Raphanus sativus* L.). Radish plants were cultivated in pots with a volume of 500 ml. Frass was applied at rates of 2 and 4 g per litre of soil. In addition to an unfertilised control, a treatment with a conventional mineral fertiliser was included for comparison. The experiment also involved a targeted infestation with the green peach aphid (*Myzus persicae*). After lyophilization, the concentrations of Na, Mg, P, S, K, Ca and Fe in the edible parts of the plants were determined using ICP-MS. The contents of these elements in radish were 0.14–1.65 (Na), 0.14–0.56 (Mg), 0.36–2.1 (P), 0.36–1.70 (S), 2.16–15.78 (K), 0.30–1.36 (Ca) and 0.005–0.124 (Fe) mg/g of fresh weight. Overall, the results indicate that frass application has a positive effect on the content of nutritionally valuable minerals in radish, and that its fertilising potential is comparable to conventional organic fertiliser. In the case of sodium (Na), a statistically significant effect of fertilisation was observed ($p = 0.04$), with the unfertilised sample showing the highest sodium content. Acknowledgement: The study was supported by the METROFOOD-CZ research infrastructure project (MEYS grant number: LM2023064), including access to its facilities.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Environmental impact of a BSF protein-based hypoallergenic dry food for dogs

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The pet food industry is actively exploring alternative, more sustainable protein sources to reduce environmental impacts while maintaining or improving pet health. Black soldier fly (BSF) derived proteins have been associated with high digestibility, hypoallergenic properties, and potential benefits for gut health and immune function in dogs. This study evaluates the environmental performance of a novel BSF-based dry hypoallergenic dog food compared with a conventional lamb-based dry hypoallergenic formulation, both produced by a commercial Portuguese pet food manufacturer and nutritionally equivalent products already commercialised. The BSF-based formulation incorporates locally sourced ingredients, including BSF protein derived from a bioconversion process in which BSF larvae valorise olive pomace, an environmental burden co-product of the olive industry. The lamb-based one relies predominantly on imported ingredients such as lamb meal. A cradle-to-gate life cycle assessment (LCA) was conducted, including ingredient production, pet food manufacturing, and packing. Primary data were collected from the BSF producer and pet food manufacturer, while background processes were modelled using Ecoinvent and Agri-footprint databases. The functional unit was defined as 1000 kcal of dry hypoallergenic dog food formulated to meet the nutritional requirements of an average adult dog. Environmental impacts were assessed using the Environmental Footprint method. Preliminary results showed that BSF-based dog food had a similar environmental impact compared to the lamb-based one. The BSF-based dog food had better results for acidification, marine eutrophication, and resource use (fossil) impact categories; the lamb-based dog food showed lower impact on climate change, eutrophication (freshwater), and photochemical ozone formation. This confirms that BSF protein can be a viable environmental ingredient for pet food. The ingredients production life cycle stage was identified as the hotspot in both systems. For instance, in climate change, BSF production makes a greater contribution due to its energy requirements; however, BSF-based dog food benefits from locally sourced ingredients. In contrast, the lamb-based formulation relied on imported ingredients, resulting in increased transport-related impacts on resource use (fossil). This work provides one of the first LCA studies of insect-based pet food in Portugal, contributing to industry-relevant evidence for the sustainable upscaling of insect-derived proteins into pet food systems.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Integrated biorefinery of insect frass for the production of biogas and artificial humic acid (AHA)

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Although edible insects have been highlighted for their ability to utilise waste as feed, insect farming itself generates a high amount of waste (frass). Frass is often proposed as a fertiliser or as a raw material for the production of biofuels and biochar. In the present study, frass was collected from the pilot-scale farming of the larvae of tree insect species: *Tenebrio molitor* (TM), *Zophobas morio* (ZM) and *Alphitobius diaperinus* (AD). This frass was evaluated for its chemical composition to explore its applicability for conversion processes to biomaterials. Afterwards, it was subjected to an integrated biorefinery, where initially it was anaerobically digested at 37 °C for 49 days with a mesophilic inoculum. After the digestion, the resulting slurry was used as the feedstock for hydrothermal humification. Alkaline-assisted hydrothermal treatment was performed using KOH, dosed according to the carbohydrate content of the digestate. Experiments were conducted on insect-derived digestates, namely TM, ZM and AD, at 180 °C for 2 h. All samples had protein content ranging from 20–25% on a dry basis and a C/N ratio ranging from 9.71-11.85. The frass from TM and ZM were richer in hemicellulose (26.95-30.82% on a dry basis respectively) and cellulose (11.23–12.13% on a dry basis respectively), while the AD frass had hemicellulose and cellulose contents equal to 18.99 ± 1.50 and 6.92 ± 1.56% on a dry basis. The digestion was implemented successfully, with the frass from TM, ZM and AD leading to a biogas yield of 602.6 ± 0.3, 539.9 ± 10.4 and 615.7 ± 0.7 L_N/kg organic dry matter, respectively, after the 49 days of digestion. The methane yield was estimated from the frass from TM, ZM and AD to be 316.4 ± 3.1, 282.8 ± 5.2 and 307.9 ± 1.2 L_N/kg organic dry matter, respectively, after the 49 days of digestion. The resulting slurry was subsequently used for the production and extraction of AHA. The process resulted in the formation of artificial humic substances and solid yields from the digestate from the frass of TM, ZM and AD that was equal to 76.1, 72.1 and 64.7% on a dry basis, respectively, while gaseous by-product formation was negligible. The successful implementation of the integrated biorefinery on insect frass and its applicability on multiple species highlights the potential of an almost zero-waste approach in insect farming, where also energy integration and crop production are enabled.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Characterisation of black soldier fly chitin: comparison of traditional chemical and subcritical fractionation

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Chitin, a non-toxic, biodegradable and biocompatible biopolymer, holds strong promise for tissue engineering and the production of renewable nitrogen-containing chemicals. Chitin is abundantly present in insects and crustaceans, yet its isolation remains challenging due to the complex structure of the exoskeleton, comprising a sclerotised network of chitin fibrils, covalently bound proteins, and embedded inorganic compounds. Conventional purification relies on lengthy (72 h) and harsh treatments with acidic, alkaline, and bleaching solutions, resulting in large amounts of hazardous waste, quality degradation and the loss of other valuable compounds. To overcome these drawbacks, we developed a novel and sustainable biorefinery process using subcritical methanol–water to isolate chitin from black soldier fly (BSF, *Hermetia illucens*) larvae in only 3 h, while simultaneously isolating other compounds such as fatty acid methyl esters. To evaluate the efficacy of this novel biorefinery process, BSF chitin obtained via subcritical fractionation (SCF) was benchmarked against BSF chitin produced by a traditional chemical process (TCP) and commercial shrimp chitin (CSC). Comprehensive characterisation included UPLC-MS and ICP-MS for purity determination; NMR and FTIR for degree of acetylation and structural analysis; WAXS for crystallinity; TGA for thermal stability; SEM for surface morphology; and colour analysis. Both BSF-derived chitins showed comparable purities (85.5% for TCP and 86.1% for SCF), while CSC exhibited a higher purity of 91.6%. Only traces of amino acids, monosaccharides, and minerals were detected in all samples, confirming efficient removal of these constituents. The degree of acetylation (FTIR) was the lowest for BSF chitin obtained via SCF (89%) and the highest for CSC chitin (99%), which was supported by NMR-based structural analysis. All samples exhibited similar crystallinity (49–54%), but differed in whitening index (54% for SCF to 89% for CSC). Thermal degradation temperatures were observed from 356 to 378 °C. Overall, these findings demonstrate that our novel subcritical fractionation biorefinery process is a viable and sustainable alternative to traditional methods for chitin isolation from BSF larvae. Despite a somewhat darker colour, the SCF process achieves comparable purity to TCP, effectively eliminates proteins, and yields chitin with physicochemical characteristics similar to those obtained via traditional extraction and commercial sources.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Enzymatic processing of black soldier fly larvae oil for sustainable polyurethane foams

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The development of sustainable, bio-based polymers is essential for advancing circular material systems. While vegetable oils are commonly used as renewable feedstocks for polyurethanes, insect-derived lipids remain largely unexplored despite their favourable sustainability profile. This study investigates black soldier fly larvae (BSFL) oil as a novel raw material for the enzymatic production of polyurethane (PU) foams. BSFL oil was converted into a polyol-rich mixture via continuous enzymatic glycerolysis using immobilized *Candida antarctica* lipase B in a packed-bed reactor at 50 °C. Reaction progress was monitored by HPLC, revealing a triglyceride conversion of approximately 85% after 240 min, accompanied by substantial formation of mono- and diglycerides suitable for PU synthesis. The resulting reaction mixtures were directly employed for PU foam preparation using diphenylmethane diisocyanate, with systematic variation of the NCO/OH ratio and polyethylene glycol (PEG500) content. The obtained PU foams exhibited densities ranging from approximately 60 to 180 kg m^{-3} and water uptake values between about 150wt% and 450wt%, depending on formulation. Increasing PEG500 content led to a pronounced increase in water uptake, reflecting the higher hydrophilicity of the foam matrix. Microcomputed tomography demonstrated highly open-cell structures with total porosities exceeding 90% of the foam volume. Thermogravimetric analysis showed the onset of major thermal degradation at approximately 310 °C, comparable to established bio-based polyurethane foams. Overall, these results demonstrate that enzymatically processed BSFL oil is a viable and sustainable feedstock for polyurethane foams, highlighting the potential of insect-derived lipids for high-value, non-food material applications within a circular bioeconomy.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

In vitro assessment of the antagonistic activity of *Tenebrio molitor* frass extracts against *Alternaria solani*

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Beyond its fertilising value, increasing evidence suggests the plant-health-promoting potential of insect frass. In this context, the present study evaluated the antifungal activity of frass derived from the yellow mealworm (YM) against *Alternaria solani*, the causal agent of early blight — a major constraint in *Solanaceae* production, particularly in tomato and potato crops. A series of *in vitro* dual-culture overlay bioassays was conducted to evaluate the effects of water-based YM frass extracts on the mycelial growth of three *A. solani* strains. Four treatments were tested: control (agar without YM frass extract), agar with 1% filtered YM frass extract, and agar with 0.1% or 1% non-filtered YM frass extract. Each treatment comprised 15 replicates. Radial growth of *A. solani* was measured after 7 and 14 days of incubation and expressed as colony diameter (mm). Similar assays were additionally conducted using a pre-commercial frass-based formulation produced via controlled biological and fermentation processes. Overall, the application of frass extracts affected fungal growth, with the magnitude and direction of the response depending on extract concentration and whether the extract was filtered. Agar amended with non-filtered frass extracts caused a profound reduction in radial growth, with the strongest inhibition at 1% non-filtered frass. In contrast, filtered frass showed little inhibitory activity and in some cases appeared to promote *A. solani* growth. The three tested *A. solani* strains differed in their responses, underscoring that intraspecific variation should be considered when evaluating control strategies. Bacterial and fungal colonies were observed only on plates amended with non-filtered YM frass, suggesting that inhibition is mediated by antagonistic interactions between *A. solani* and the frass-associated microbiome. Initial microbiome screening using MALDI-TOF MS identified *Aspergillus flavus*. Although an *A. flavus* strain (PA67) has been reported as a potential biocontrol agent against several fungal pathogens, its relevance for *A. solani* remains unclear. Further work will aim to characterise the complex microbiome involved and to elucidate its role in plant pathogen suppression.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Isolation and *in vitro* characterisation of plant growth–promoting bacteria associated with black soldier fly frass

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Hermetia illucens (black soldier fly, BSF) is an efficient insect for the bioconversion of organic residues into valuable larval biomass. Among insect farming by-products, larval frass has gained attention for agricultural use due to its nutrient content and association with plant growth–promoting bacteria (PGPB), both strongly influenced by larval diet. In accordance with EU Regulation 2021/1925, frass must be subjected to heat treatment at 70 °C for 1 h to reduce the presence of pathogenic microorganisms such as *Salmonella* spp. and *Escherichia coli*. Frass derived from larvae reared on different feeding substrates (vegetable waste, olive pomace, dairy residues, spent malt, and a standard Gainesville diet) was microbiologically analysed before and after heat treatment, with particular attention to the PGPB diversity and functional potential. Bacterial isolates screened *in vitro* for key plant growth–promoting traits, including tolerance to humic acids, phosphate solubilization capacity, ammonia release, and auxin and gibberellin production. Microbial analyses highlighted a substantial presence of PGPB in BSF frass. A total of 149 bacterial isolates were recovered, of which 73 with positive PGPB traits were identified through 16S rRNA sequencing. Members of the families Moraxellaceae and Enterobacteriaceae were predominant in both untreated and heat-treated samples, indicating limited effects of thermal treatment, while substrate strongly shaped community composition. Six strains with high functional potential belonging to the genera *Serratia*, *Peribacillus*, *Acinetobacter*, *Pseudocitrobacter*, *Bacillus* and *Enterobacter* were tested on *Arabidopsis thaliana* seeds to assess their effects on plant development. The *in vivo* experiments demonstrated strain-specific influences on seed germination, primary root growth, and root hair formation. Overall, the findings demonstrate that BSF frass harbours a metabolically active microbial community that is partially resilient to thermal treatment. This by-product represents a promising resource for the development of biofertilisers, and the strategic selection of larval diets may enable the production of frass with enhanced functional properties while maintaining microbiological safety.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Fortified bread with domestic cricket (*Acheta domesticus*) and marigold flower

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Bread is one of the most widely consumed foods worldwide and serves as an effective vehicle for incorporating functional ingredients. For young consumers seeking a balanced and sustainable diet, a bread enriched with edible flowers and insects could be a option. This additions not only provide bioactive compounds but also aide with environmentally conscious choices. This study aimed to optimise the formulation of a wheat bread fortified with domestic cricket (*Acheta domesticus*) flour and marigold (*Tagetes erecta* L.) flower. A D-optimal mixture design was carried out using Design Expert 13 software, varying the proportions of wheat flour (0–100%), cricket flour (0–15%), and marigold flower (0–5%). Thus, 11 formulations were generated and evaluated for their physical properties (colour, height, specific volume, and weight), textural properties, total carotenoid concentration, moisture, protein content, and *in vitro* protein digestibility. Using response surface methodology, two optimal formulations were obtained that minimised the impact of adding cricket flour and marigold flower on the physical properties of wheat bread. Compared with the control product (100% wheat flour), the optimised breads exhibited decreases in height (9–15%) and specific volume (3–29%). On the other hand, product weight increased (6–9%), and darker, slightly yellow crumbs and crusts were obtained. Regarding textural parameters, bread hardness showed a slight decrease (1–3%), while the other textural attributes followed the same trend. In terms of protein, the optimised breads increased their protein content (3–10%); however, their digestibility showed a slight decrease (approx. 5%). Finally, these products exhibited an increase in total carotenoid concentration (417–590%). The fortification of wheat bread with cricket flour and marigold flower represents a viable alternative to enhance its protein content and functional profile; however, some of its physical characteristics are compromised. Therefore, it is important to conduct a sensory analysis of the optimised products to evaluate consumer acceptance levels.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Effect of black soldier fly larvae frass on growth and phenolic content of salad crops in pot and field trials

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There is a growing interest in alternative nutrient sources that can reduce the reliance on synthetic fertilisers, while maintaining crop productivity and quality. Among these, black soldier fly larvae (BSFL) frass has gained recognition as organic fertiliser and soil amendment. It consists of undigested substrates, larval faeces and chitin-containing exudates and is rich in essential plant nutrients as well as phytohormones and beneficial microorganisms. These characteristics suggest, in addition to fertilising properties, a possible biostimulant effect that can influence plant metabolism and response to stress factors. The aim of this research is to investigate the effect of different BSFL frass variants on plant growth and the changes in secondary metabolite profiles. Pot and field trials were conducted on salad crops. Pot trials were performed using the variety Suzan under controlled conditions (22 °C day/18 °C night, 16-h photoperiod, 100 μmol/m^{2}s, 70% relative humidity). Different BSFL frass variants (e.g. originating from different feed mixes or frass that were differently processed) were applied at the recommended nitrogen rate of 115 kg/ha. Organic and synthetic fertiliser controls, as well as unfertilised control, were included. At harvesting, plant growth parameters including leaf number, plant dry weight and roots length were measured. Field trials were conducted on Iceberg lettuce to evaluate the potential biostimulant effect of frass applied in combination with synthetic fertiliser at a nitrogen rate of 112 kg/ha. Its performance was compared to chicken manure supplemented with synthetic fertiliser and synthetic fertiliser alone. Harvested leaves were lyophilized and analysed for total phenolic content (TPC) using the Folin–Ciocalteu mehod, while total leaf nitrogen content was measured by Dumatherm. Pot trial results indicated a positive growth response to frass application, with yields and root lengths comparable to those obtained with digestate used as benchmark organic fertiliser. The field trials showed up to 40% increase in the yield compared to chicken manure and up to 16% compared to only synthetic fertiliser. Overall, the study underpins the positive fertilising effect of insect frass for salad crops, while its effect on changes in composition of secondary metabolite still remains to be further analysed.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Microbial community shifts in maize rhizosphere in response to black soldier fly frass under Indian black soil

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Frass from black soldier fly (BSF) (*Hermetia illucens* L.) based waste bioconversion is being recognized as a promising soil amendment due to its nutrient profile and biologically active components. However, Field based evidence characterising the effects of frass application on soil microbial biomass, their interaction with native microflora is still limited especially under cropping systems in Indian soils. The field experiment has been carried out with four treatments comprising of Municipal solid waste (MSW) derived BSF frass without heat treatment and MSW derived BSF frass with heat treatment (Regulation (EU) 2021/1925), conventional fertiliser and control, each having three replicate plots. Recommended dosage of conventional fertiliser and Nutrient equivalence of BSF fertiliser has been applied at the time of sowing. The microbial community structure and functional diversity in the rhizospheric soil has been assessed using whole genome metagenomics analysis through nanopore sequencing at the grand growth stage of the crop. The results revealed that BSF frass which has undergone thermal treatment has outperformed other treatments in maintaining rhizospheric microbial integrity with native microflora and have added advantages over other treatments. The beta diversity analysis showed the increased diversity in BSF treatments and a decline in conventional fertiliser treatment over control. Microbial genera known to enhance the plant growth through PGPR production, nutrient cycling and mobilization has been found in both BSF frass treatments. Our findings validate the practical significance of using BSF frass with and without heat pre-treatment in crop cultivation alongside maintaining microbial safety.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Biofertilising potential of *Hermetia illucens* frass in field-grown maize

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Mineral fertilisers have long been widely used in agriculture. However, rising energy prices reduce the economic feasibility of their production and application, while excessive use negatively affects biodiversity and soil health and often results in low nutrient use efficiency by plants. Consequently, interest in alternative organic nutrient sources has increased. During the rearing of black soldier fly *Hermetia illucens*) larvae, a by-product known as frass is generated, which has the potential to be used as an organic fertiliser for agricultural crops. This field study, conducted during the 2024–2025 growing seasons, evaluated the agronomic performance of *H. illucens* frass obtained from larval production, pelletized, and applied to maize hybrid Blackrock. All plots received a uniform basal fertilisation with urea at 150 kg ha^{-1} (69 kg N ha^{-1}). In the experimental treatment, frass was additionally applied at 7.5 t ha^{-1} (dry matter 85.6%) to evaluate its fertilising potential. Plant density was 88 000 plants ha^{-1}. Both control and frass-treated plots were arranged in a randomised design with three replicates, and each plot covered an area of 50 m^2. The experimental site was located at 49 °52′50.4″ N, 24 °12′29.8″ E. Plant height was measured at three growth stages: 8–10 leaf stage, flowering stage, and prior to grain harvest. At the 8–10 leaf stage, the average plant height in control plots was 45.83 ± 6.91 cm in 2024 and 72.12 ± 4.20 cm in 2025, whereas frass-treated plants reached 60.27 ± 3.08 cm and 84.18 ± 2.99 cm, respectively. During the flowering stage, plant height in control plots was 172.53 ± 15.65 cm (2024) and 109.63 ± 8.40 cm (2025), compared to 211.48 ± 9.73 cm and 126.10 ± 2.09 cm in the frass-treated plots. At harvest, maize plant height in the control treatment reached 234.18 ± 12.39 cm in 2024 and 287.50 ± 9.54 cm in 2025, while plants fertilised with frass were taller, reaching 262.62 ± 9.12 cm and 302.63 ± 4.16 cm, respectively. The overall greater plant height observed in 2025 can be attributed to higher precipitation during the growing season. A similar trend was observed for grain yield. Average yield in the control treatment amounted to 13.82 ± 0.53 t ha^{-1} in 2024 and 12.54 ± 1.24 t ha^{-1} in 2025, whereas frass application resulted in yields of 15.80 ± 1.15 t ha^{-1} and 13.36 ± 0.32 t ha^{-1}, respectively. Overall, the results demonstrate that frass used as an organic fertiliser enhances plant growth and grain yield of maize under field conditions and represents a promising component of sustainable fertilisation strategies.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Growing corn using house cricket frass as a fertiliser

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The rapidly developing insect industry produces not only insects but also substantial amounts of frass, a mixture of insect faeces, exuviae, dead insects, and residual substrate. Frass can contain essential nutrients and bioactive compounds, such as chitin and beneficial microorganisms, giving it potential as both an organic fertiliser and biostimulant. Its use as a soil amendment could enhance the sustainability and circularity of edible insect production. However, while frass from black soldier fly larvae and mealworms has been studied, data on cricket frass remain scarce. Therefore, this study evaluated the fertiliser potential of house cricket (*Acheta domesticus*) frass in a model pot experiment. Its effects were compared with a commercial NPK fertiliser and vermicompost. All treatments were applied at two rates of frass (72 g and 144 g per 4.5 kg of soil). Hybrid corn RGT Attraxxion was used as the model plant, and the trial lasted 15 weeks, and the elemental composition of harvested corn biomass was determined using ICP-OES and a CHNS elementary analyser. The results showed the most substantial differences in the case of nitrogen contents in corn plants, where the average N contents in frass treated variants were 1.01 and 0.88% in stems and leaves, respectively, for the lower frass application rate, and after the higher application rate the N contents in plants increased by 50% in stems and 35% in leaves compared to the lower rate. These values were significantly ($p < 0.05$) higher compared to both control and vermicompost variants. However, the NPK variant reached up to 4% in stems and 2.6% in leaves, documenting slow release of N from the organic fertilisers compared to NPK. On the contrary, for micronutrients (Fe, Mn, Cu), as well as for K and Mg, no significant differences were observed among all the variants. Overall, house cricket frass shows promise as an alternative organic fertiliser. Future studies should assess its performance in different crop species and clarify its biostimulatory effects under controlled and field conditions.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Chitosan from black soldier fly puparium: extraction, characterisation and perspectives on substrate-driven variability

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The insect industry has grown exponentially in recent years, with species such as the black soldier fly (BSF, *Hermetia illucens*) playing an important role in sustainable animal feed production and agro-industrial byproducts management. A by-product of BSF rearing is the puparium, a cocoon-like exoskeleton left after the fly emerges, a valuable source of chitin, which may be converted into a versatile application material, chitosan. Chitosan is a biomaterial with great potential for biomedical applications with antioxidant, antimicrobial, anti-inflammatory and healing effects. This study aims to obtain and characterise the chitosan produced from BSF puparium envisaging its use in veterinary applications. For this purpose, a chemical extraction protocol was implemented to isolate chitin from BSF puparia through sequential steps of demineralisation, deproteinization and depigmentation. Chitosan was subsequently produced through repeated deacetylation cycles, hydrolysis and purification. Its physicochemical properties were assessed by spectroscopic techniques and scanning electron microscopy. The obtained chitosan exhibited a degree of deacetylation of approximately 70% and an average molecular mass of around 200 kDa. In parallel, this work is being extended to investigate the influence of larval rearing substrates on the properties of chitosan derived from BSF puparium. Comparative analyses between chitosan's obtained from puparia originating from different feeding substrates are currently underway, and preliminary trends will be discussed where available. Additionally, the potential antimicrobial activity of chitosan was also carried out *in vitro* using *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538 as model microorganisms. Notably, the chitosan obtained in this study has already demonstrated antimicrobial activity against both microorganisms tested. Acknowledgements: This study is under the scope of a FCT PhD scholarship UI/BD/154566/2023 (https://doi.org/10.54499/UI/BD/154566/2023) and a CiiEM Research Grant RG 16_24 "Chitosan gel produced from Black Soldier Fly exuviae for applications in oral and teeth health – ChiTeeth".

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Techno-functionality of insect lecithin in dark chocolate

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In this study, we investigated the potential of yellow mealworm (YMW) and black soldier fly larva (BSFL) lecithin as rheology-modifying alternatives to soy lecithin in dark chocolate. We examined the impact of varying concentrations of insect lecithin on the flow properties of molten chocolate, including Casson viscosity and yield stress, which are critical for processes such as molding and tempering. Suitable lecithin concentrations for achieving comparable Casson viscosity to soy lecithin are identified. Additionally, the influence of these rheological properties on the physical characteristics, texture, and appearance of solidified chocolate discs is assessed. Sensorial attributes such as colour, appearance and aroma were instrumentally assessed. Rheological assessments highlighted that insect lecithin, particularly from BSFL, exhibited comparable or superior viscosity-reducing capabilities, suggesting its potential as a natural alternative to traditional lecithin sources. This behaviour was related to the distinct phospholipid profiles across the lecithin sources, which were previously reported. Lecithin concentrations of 0.3% for BSFL and 0.6% for YMW were determined as suitable for modifying Casson viscosity in dark chocolate. Both presented effects comparable to 0.3% DSL, with BSFL lecithin notably maintaining yield stress without compromising viscosity. Additionally, the melting profiles indicated stable chocolate products with overall similar texture and color properties, showcasing that insect lecithin performs effectively in achieving desirable chocolate characteristics. The aroma profile analysis highlighted the distinct contributions of insect lecithin to chocolate aroma. Notably, YMW lecithin enhanced specific desirable aroma compounds, such as ethyl butyrate, which may be partially attributed to its higher concentration (0.6%) compared to BSFL and soy lecithin (0.3%). Meanwhile, BSFL lecithin produced similar aroma profiles to those of soy lecithin, without significantly detracting from the overall sensory quality of the chocolate. In conclusion, the results suggest that insect lecithin, especially from BSFL and YMW, can serve as viable alternatives to soy lecithin in chocolate formulations, potentially offering both functional benefits and sensory enhancements. This study contributes to the broader goal of establishing insect lecithin as a sustainable and functional food ingredient. By providing critical insights into its techno-functional properties in chocolate, this work lays the foundation for future research and innovation in sustainable ingredient applications.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Derivatives of *in vitro* digestion and fermentation of chitin extracted from field cricket, house cricket and BSF

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Increase in microbial resistance has prompted evaluation of bioactive biopolymers such as chitin. Edible insects' chitin is an untapped resource since edible insects have a high biodiversity and considerable amounts of chitin. Thus the aim of this study was to investigate the derivatives obtained following *in vitro* digestion and fermentation of edible insects' chitin. Chitin was chemically extracted from *Acheta domesticus*, *Gryllus bimaculatus* and *Hermetia illucens* then subjected to *in vitro* digestion. The indigestible chitin was then subjected to in-vitro fermentation using probiotic starter cultures i.e. ABY10 and ABT 5. Short Chain Fatty Acids (SCFA), chitosan, chito oligosaccharides, antimicrobial peptides (AMP) and vitamins were then quantified. Close to three quarters of the total fatty acids were SCFA followed by Lauric acid and then trace amounts of other saturated and unsaturated fatty acids. The predominant SCFA was acetic acid, followed by propionic acid as well as trace levels of 4 methyl valeric acid, isovaleric acid and valeric acid. The highest values of chitosan were obtained after fermentation of *Acheta domesticus* indigestible chitin using ABY 10 and ABT 5 for 48 hours (2.91 g/100 g and 2.90 g/100 g) and fermentation of *Hermetia illucens* indigestible chitin using ABY 10 for 48 hours (2.90 g/100 g). *Gryllus bimaculatus* indigestible chitin fermented using ABY 10 for 48 hours and *Acheta domesticus* indigestible chitin fermented using ABY 10 and ABT 5 for 48 hours had the highest chito oligosaccharides yield. *Gryllus bimaculatus* indigestible chitin fermented using ABY10 for 48 hours, *Acheta domesticus* and *Hermetia illucens* indigestible chitin fermented using ABY 10 for 48 hours yielded the highest defensin like AMP. *Gryllus bimaculatus* and *Hermetia illucens* indigestible chitin fermented using ABY 10 for 48 hours and *Hermetia illucens* indigestible chitin fermented using ABT 5 for 48 hours yielded the highest vitamin A values. The highest vitamin B2 content was yielded after fermentation of *Gryllus bimaculatus* and *Acheta domesticus* indigestible chitin fermented using ABY 10 for 48 hours (2.17 mg/100 g and 2.14 mg/100 g). *Gryllus bimaculatus* indigestible chitin fermented using ABY 10 for 48 hours yielded the highest vitamin B12 content (5.74 mg/100g). In regards to fermentation time 48 hours seemed to be optimal. The study findings thus indicate that chitin inclusion in food products or consumption of whole insect could potentially promote gut health.

IMPACT AND APPLICATIONS OF INSECT-DERIVED PRODUCTS

Effect of black soldier fly frass fertiliser on selected soil properties and yields of tomatoes in a degraded ferralsol

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Declining soil health poses a major challenge to vegetable production in Uganda. Organic inputs such as black soldier fly (BSF) frass fertiliser have been reported to improve soil health and crop productivity; however, empirical evidence under Ugandan conditions remains limited. This study evaluated the effects of BSF frass derived from poultry droppings (PD) and from a mixture of poultry droppings, cow dung and jackfruit waste (PCJ), relative to inorganic NPK fertiliser, on soil chemical and microbial properties, tomato growth, and yield. The experiment was conducted using a randomised complete block design (RCBD) with six treatments: PD frass, PCJ frass, PD + NPK, PCJ + NPK, NPK, and no fertilisation (control). Fertiliser application rates of 11.7 t/ha (PD), 10.9 t/ha (PCJ) and 2.2 t/ha (NPK) were based on a tomato nitrogen requirement of 371 kg/ha and applied in two split doses over a 13-week experimental period. Results showed that PD frass produced the highest and most significant increase in arbuscular mycorrhizal fungal spore numbers ($p = 0.003$) and improved soil chemical properties, while *Pseudomonas fluorescens* presence increased in PCJ + NPK treatment and Trichoderma in both PD and PCJ + NPK treatment. Tomato yield was significantly affected by fertiliser treatment (ANOVA: $F_{5,12} = 3.93$, $p = 0.024$). PD produced the highest mean yield (19.5 t/ha), followed by PCJ (18.1 t/ha), PCJ + NPK (17.7 t/ha), PD + NPK (15.4 t/ha), control (9.7 t/ha) and NPK alone (8.0 t/ha). Yield differences between PD and PCJ were not statistically significant. Overall, BSF frass applied alone or in combination with NPK enhanced soil health indicators, rhizosphere functioning, and tomato productivity compared with inorganic fertiliser or no fertiliser input. These findings highlight the potential of BSF frass as an effective organic soil amendment for sustainable tomato production in Uganda, although further research is required to assess long-term effects on soil microbial communities and soil functioning.

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Black soldier fly larvae in slow-growing chicken diets: effects on growth, gut health and digestive enzyme modulation

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Black soldier fly larvae (BSFL) supplementation in chicken diets has been proposed as a sustainable protein source rich in nutrients. This study evaluates the impact of supplementing dehydrated and live BSFL reared on Gainesville substrate (D-BSFL and L-BSFL, respectively) to a slow-growing chicken breed called Bianca di Saluzzo (BS). A total of 144 BS males (39 days old) were distributed in 18 pens (3 treatments/6 replicates). The groups were categorized as: (1) Control Group (C) fed a basal diet (0% soybean meal substituted with alternative protein ingredients); (2) DL group fed the C diet supplemented with 5% D-BSFL; (3) LL group fed the C diet supplemented with 5% L-BSFL. The larvae consumption time was recorded weekly. Feed intake was recorded weekly and larvae were provided at 5% of dry matter intake. Birds were weighed and feed conversion ratio calculated every 21 days. At 147 and 174 days, two birds per replicate (72 total) were slaughtered. Five-cm segments of the duodenum, jejunum, ileum, spleen and left liver lobe were collected for histological and histopathological evaluation. Colloidal chitin was incubated with proventriculus enzyme extracts to assess endo-, exo-chitinase, and chitosanase activities. Levene's test was used to assess variance homogeneity, considering pens for growth performance and individual birds for gut analyses. Data were analyzed using a Generalized Linear Model (RStudio). DL and LL groups showed higher feed intake and 4% higher final live weights compared to C (C: 2335 g; DL: 2440 g; LL: 2412 g). However, no differences were noted in FCR among the groups. The supplementation of BSFL did not highlight alterations in intestinal morphometry or histopathological severity in any of the sampled organs confirming the safety of BSFL supplementation for chickens. Additionally, animals fed BSFL showed markedly higher endo-chitinase and exo-chitinase activities than controls (+96.9% and +48.2%, respectively), whereas chitosanase activity remained unchanged, indicating that BSFL inclusion selectively modulates chitinolytic enzyme activity. In conclusion, the birds demonstrated good adaptability in response to the supplementations of BSFL, with no differences at gut level between live or dehydrated larvae. Overall, such adaptive responses underscore the capacity of slow-growing breeds to adjust and optimize their dietary strategies in response to alternative feeding regimens, appearing particularly suitable for extensive and slow-growing production systems, where birds' adaptive capacity and longer rearing periods allow full nutritional and physiological benefits to emerge.

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Effect of dietary inclusion of camelina and linseed cakes on the amino acid content of *Tenebrio molitor* larvae

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Oilseed by-products are increasingly considered for use in insect feeding substrates to support circular economy and the valorisation of agri-food residues. While the effects of oilseed cakes on the lipid profile of insects appear to be relatively direct, their impact on protein deposition and amino acid content remains largely unexplored. This study evaluated the effects of dietary inclusion of camelina (*Camelina sativa*) and linseed (*Linum usitatissimum*) cakes on the amino acid content of *Tenebrio molitor* (TM) larvae. Five-week-old larvae were randomly allocated to six dietary treatments: a standard farm diet (STD); a control diet (CON); CON including 5% camelina cake (CAM 5); 10% camelina cake (CAM 10); 5% linseed cake (LIN 5); or 10% linseed cake (LIN 10). Except for STD, all diets were formulated to be isoprotein and isoenergy. Each treatment included 12 replicates. Larvae were fed *ad libitum* and reared for four weeks under controlled environmental conditions until 9 weeks of age. At the end of the trial, larvae were collected and analysed at LabCNX of MAPS Department for amino acid composition. Data were analysed using one-way ANOVA. Dietary inclusion of camelina and linseed cakes significantly affected both essential and non-essential amino acid contents of TM larvae ($P < 0.0001$). Larvae fed CAM 5 and LIN 5 diets showed higher contents of several essential amino acids such as histidine, isoleucine, threonine and valine, compared with the other treatments ($P < 0.0001$). Conversely, increasing the inclusion level to 10% resulted in a reduction of total amino acid content and of key essential amino acids, particularly lysine and methionine ($P < 0.0001$). Non-essential amino acids, including proline, serine and tyrosine, followed a similar trend, with higher concentrations at 5% inclusion compared with both CON and standard STD diets, and reduced values at 10%. Glycine showed an opposite trend, with the highest value observed in the LIN 10 group (933 vs 652 mg/100 g, on average). Linseed cake at 5% inclusion led to the highest total amino acid content in larvae (14 058 mg/100 g, as is) followed by CAM 5 group (13 439 mg/100 g, as is). These results indicate that TM larvae can modulate their amino acid profile in response to dietary composition and that moderate inclusion levels (5%) of camelina and linseed cakes may enhance amino acid deposition, whereas higher inclusion levels (10%) may impair protein utilisation. The findings support the use of oilseed by-products at low inclusion levels as sustainable ingredients in TM rearing substrates.

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Fermentation metabolite dynamics during black soldier fly larval rearing

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Fermentation of substrates is a common pre-treatment in black soldier fly larvae (BSFL) bioconversion. However, little is known about substrate fermentation occurring during larval rearing, despite the ideal conditions for microbial growth during BSFL bioconversion. This study characterised temporal changes in fermentation metabolites in a high-moisture substrate (73% moisture, 27% dry matter) during BSFL rearing and evaluated impacts on substrate nutrient availability and microbial activity under larval influence. Larvae were reared on an experimental substrate (whey, wheat bran) under controlled rearing conditions in stainless steel trays (2.68 × 1.12 m). Each tray contained 260 000 larvae and 180kg feed. Ambient temperature was 30 °C, relative humidity 70%, and initial substrate temperature 28 °C. Feed samples were collected daily at 14:00 on days 0, 2, 5 and 6 and analysed for key fermentation metabolites using including lactic acid, ethanol, 1-propanol, and 1,2-propanediol. Total solids were determined gravimetrically (VDLUFA III, 3.1, 1976), while organic acids and alcohols were quantified using internal chromatographic and gas-chromatographic methods (LKS FMUAA 166:2024-11 and 181:2024-11). Metabolite concentrations were normalized to dry mass to assess temporal dynamics during active larval feeding. Fermentation metabolite profiles changed markedly over the rearing cycle. Data were analysed using ANOVA with post-hoc testing ($p < 0.05$). Lactic acid dominated the metabolite spectrum and increased substantially during the first 3 days of rearing with an observed increase of approximately 20% between day 2 and day 3. Alcohols such as ethanol and 1-propanol were present at lower concentrations (<15% of total fermentation metabolites). Ethanol reached its highest concentrations during early rearing (day 2) and decreased thereafter, while 1-propanol remained at consistently low levels (<2%) across all sampling days. These dynamics indicate shifts in the substrate microbial metabolism during rearing, consistent with reported changing redox conditions driven combined larval and microbial respiration, substrate turnover and larval movement. Thus, temporal patterns in fermentation metabolites, particularly lactic acid, may therefore serve as indicators of microbial activity and metabolic state during insect rearing. The results further demonstrate that nutrient concentrations in the rearing substrate change dynamically during bioconversion, such that initial substrate values are unlikely to represent nutrient availability at peak larval demand. Nutrient availability during BSFL rearing should thus be considered a dynamic process rather than a static substrate property.

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Insects can bioaccumulate phytochemicals from olive oil by-products

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Research on the functional properties of insects, such as *Hermetia illucens* (HI) or *Tenebrio molitor* (TM) is growing, evidencing their potential as novel health-related foods. Their bioactive composition and derived bioactivity seem to be influenced by diet, particularly when insects are fed vegetable ingredients rich in bioactive phytochemicals. In this context, feeding bioactive-rich agri-food by-products could valorise side streams while enriching insects with phytochemicals, although evidence on the effective bioaccumulation of specific compounds and related bioactivities remains limited. This study aimed to evaluate the effect of feeding HI and TM larvae with different levels (15–90%) of bioactive-rich by-products well-known from the olive oil industry (olive leaves or olive pomace) on the phytochemical bioaccumulation and antioxidant activity of insect meals. Reared larvae were slaughtered, dried, ground, and defatted. Meals were characterised for target olive phytochemicals (GC-MS previous silylation or HPLC-DAD) following 70% ethanol ultrasound-assisted extraction. The efficiency of bioaccumulation (EB,%) of phytochemicals was estimated as the proportion of the ingested compound accumulated relative to the theoretical maximum. Antioxidant activity was evaluated (DPPH method). Insects accumulated most targeted dietary phytochemicals, which were not detected in control larvae. However, despite diets being richer in phenolic compounds such as oleuropein (olive leaves) and hydroxytyrosol (olive pomace), together with minor levels of pentacyclic triterpenes, the insect meals mainly accumulated pentacyclic triterpenes, particularly oleanolic and maslinic acid (HI, TM) and ursolic acid (TM). EB values (0.6-3.5% for HI; 0.4-1.8% for TM) suggested limited retention of the phytochemicals. Nevertheless, the achieved enrichment was relevant enough considering the absolute accumulated content of phytochemicals. In fact, the total amount of phytochemicals in the meals strongly correlated with antioxidant activity. This study evidences the possibilities of dietary strategies to enrich insects with bioactive phytochemicals, enabling the use of insects as bioactive carriers and enhancing their health-related properties, while supporting circular economy approaches through by-products valorisation. Acknowledgment: ENTOMOTIVE project, PID2022-136238OB-I00 and grant PREP2022-000828, funded by MICIU/AEI/10.13039/501100011033 and ERDF/EU.

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In vitro assessment of immunomodulatory and antimicrobial mechanisms of black soldier fly larvae derivatives

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Optimised nutrition is a key strategy to support animal health in both companion animals and farmed species such as aquaculture. Pets commonly suffer from chronic inflammation, while farmed animals are exposed to stress and pathogens inducing pro-inflammatory conditions and increased mortality. In this context, black soldier fly larvae (BSFL)-derived ingredients have been shown to support health and, in some cases, improve survival. However, although compounds such as antimicrobial peptides, lauric acid and chitin are recognized for their immune properties, the underlying mechanisms remain unclear. In this work, we investigated two complementary aspects of BSFL-derived ingredients' health benefits: (i) the immunomodulatory potential of a soluble BSFL hydrolysate extract (BSFL-HE) using a canine whole blood model and cytokine secretion analysis, and (ii) the direct bacterial inhibition potential of the same extract against several aquaculture-relevant bacterial strains. Whole blood from male Beagle donors was pre-incubated with BSFL-HE for 6 hours, followed by induction of a pro-inflammatory state using LPS. After 24 hours, plasma concentrations of IL-6, IL-10, and TNFα were quantified via a multiplexed magnetic bead-based assay. Dexamethasone was used as a positive anti-inflammatory control and inhibited the secretion of all cytokines. BSFL-HE reduced secretion of the pro-inflammatory cytokine TNFα while increasing secretion of the anti-inflammatory cytokine IL-10, indicating an anti-inflammatory effect in an LPS-induced inflammatory context. BSFL-HE also exhibited biphasic control of IL-6 secretion, with a specific dose consistently suppressing its expression. To evaluate antibacterial activity, bacterial growth in the presence of increasing concentrations of BSFL-HE was monitored by turbidity measurements at OD600 and normalized to untreated and florfenicol-treated controls. BSFL-HE inhibited the growth of *Aeromonas salmonicida*, *Flavobacterium psychrophilum* and *Photobacterium damselae* at the highest tested concentrations. Together, these results suggest that BSFL-derived ingredients may support animal health through complex immune-related mechanisms, combining direct bacterial growth inhibition with modulation of inflammatory responses and cytokine production. These findings may help explain *in vivo* observations of improved immune status in animals fed BSFL-based ingredients, although further studies are required to confirm the effects of BSFL-HE when administered as part of a complete diet.

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Feeding live black soldier fly larvae to free-range Lohmann Brown laying hens: effects on performance and egg quality

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Egg production faces increasing demands to improve sustainability, especially by reducing reliance on imported protein sources like soybean meal. This study investigated the effects of feeding live black soldier fly larvae (LBSFL) as a substitute for soybean meal (SBM) and soybean oil (SO) on the laying performance, egg quality traits, and fatty acid composition of the egg yolk of Lohmann Brown laying hens. A total of 144 18-week-old hens were allocated equally (4 pens/treatment, 18 hens/pen, free-range system) to two dietary treatments: SOY and LAR. Birds in the SOY group received a commercial laying diet with 15% SBM+SO during phase 1 (P1, 18 – 39 weeks) and 10% SBM+SO in phase 2 (P2, 40 – 50 weeks), while SBM+SO was completely replaced by LBSFL in LAR treatment. LBSFL was offered twice a day based on expected dry matter intake. Growth performance and feed intake were recorded weekly, laying performance daily, and egg quality weekly (P1) or every two weeks (P2). Yolk fatty acid composition was analyzed at the end of each phase. Data were analysed using one-way ANOVA ($p < 0.05$). Feeding LBSFL did not affect hens' growth and laying performance ($p > 0.05$). During the whole trial period, BSFL-fed hens showed a significantly reduced overall dry matter intake ($p < 0.05$) and a higher average individual egg weight ($p < 0.05$), resulting in a tendency toward improved feed conversion ratio ($p < 0.1$). During the entire trial period, there were no significant differences in the egg quality parameters such as shape index, Haugh units, amount of yolk, pH value in yolk and albumen, and yolk index between both treatments (n = 2296). However, individual egg weight and yolk colour ($p < 0.05$) increased significantly, while shell thickness ($p < 0.05$) and amount of shell weight ($p < 0.05$) were reduced in LAR. LBSFL feeding increased the saturated fatty acid (SFA) content and reduced the polyunsaturated fatty acid (PUFA) proportion in LAR-yolk, compared to SOY, while total FA and monounsaturated FA (MUFA) were unaffected. The increase in SFA was primarily due to higher levels of C12, C14 and C16:0, which are the main FAs present in BSFL. The results of this study indicate that replacing SBM+SO with live BSFL is a suitable and sustainable alternative protein and energy source for laying hens, while maintaining production performance and modifying egg yolk FA composition.

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Looking beyond nutrients: NMR metabolomics on edible insects for a further understanding of their chemical profile

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Edible insects are becoming even more a nutritional source and a valid protein alternative to the conventional ones (Mariutti *et al.*, 2021). This great interest has caused in the last years an increase in studies aimed at the valorisation of their nutritional profile and applications. In this context, attention has been mainly focused on the main macro- and micro-nutrients (Kouřimská and Adámková, 2016). On the contrary, a lack of studies aimed to characterise the metabolite profile of insects exists, although these molecules can be involved in both nutritional and biological activities. Moreover, the metabolite profile is strictly correlated with the phenotype of biological matrices, representing a valid parameter to monitor the effects of several variables involved in production, preservation, and processing. Here, several examples of NMR metabolomics applied on edible insects are reported, with different conditions considered in each study. In the first one, an NMR metabolomic analysis of three species (*A. domesticus*, *T. molitor*, *L. migratoria*) reared in the same conditions was carried out. Moreover, the same samples were subjected to an *in vitro* digestion process to observe the release of nutrients and other metabolites. In a second study, *T. molitor* was reared using different percentages of food waste chestnut shell and analysed by means of NMR spectroscopy (Ferri *et al.*, 2024a,b). Finally, another study considered the effect of growth stage and drying processes on *A. domesticus* samples. The obtained results underlined, for each case study, a profile characterised by metabolites involved in nutritional but also biological activities, highlighting potential roles of edible insects in human health. Moreover, the application of the described variables caused significative changes from a qualitative and quantitative point of view, conversely to what was often observed when macronutrients were considered in the same samples. NMR metabolomics showed to be a potential tool to obtain a "larger view" of edible insects chemical profile, giving a further valorisation and a better understanding of their composition and uses. At the same time, the monitoring of metabolite profile confirmed to be a strong strategy to observe the response of edible insects to production and technological practices, contributing to the development of standardized practices to obtain products with specific characteristics. References: Ferri, I., Dell'Anno, M., Spano, M., Canala, B., Petrali, B., Dametti, M., Magnaghi, S. and Rossi, L., 2024a. Characterisation of *Tenebrio molitor* reared on substrates supplemented with chestnut shell. Insects 15: 512. Ferri, I., Spano, M., Dell'Anno, M., Mannina, L. and Rossi, L., 2024b. Metabolomic Profiling of *Tenebrio molitor* reared on chestnut shell-enriched substrate using NMR Ppectroscopy. Foods 13: 3757. Kourimská, L. and Adámková, A., 2016. Nutritional and Sensory Quality of Edible Insects. NFS Journal 4: 22-26. (3) Mariutti, L.R.B., Rebelo, K.S., Bisconsin-Junior, A., Santos de Morais, J., Magnani, M., Rodrigues Maldonade, I., Madeira, N.R., Tiengo, Maróstica Jr, M.R. and Cazarin, C.B.B., 2021. The use of alternative food sources to improve health and guarantee access and food intake. Food Research International 149: 110709.

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Refining protein quantification in mealworm and cricket meals: impact of processing on K_p and K_A factors

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Conflicting values have been reported for the nitrogen-to-protein conversion factor (K_p) used to estimate protein content from total nitrogen (N_{total}) in edible insects, complicating accurate protein estimation. This work aims to demonstrate that protein concentration in insect meals can be determined more reliably by applying the K_A conversion factor to protein-bound nitrogen (N_P). Cricket (*A. domesticus*) and mealworm (*T. molitor*) meals were prepared from insects treated by various killing (freezing, blanching) and drying (freeze-drying, oven-drying, roasting) methods. Amino acid profiles of insect meals were obtained by UPLC, and K_p and K_A factors were calculated. Non-protein and non-chitin nitrogen ($N_{NP,NC}$) was quantified through protein precipitation using tricholoracetic acid and acetone. Chemical deproteinisation (1 M NaOH, 75 °C, 60 min) was then performed, followed by filtration to recover N_P. Nitrogen content in all nitrogenous fractions (N_{total}, $N_{NP,NC}$, N_P, N_{other}) were measured using the micro-Kjeldahl method. Crickets killed by freezing exhibited significantly lower K_p factors (3.11–3.69) than those subjected to blanching (4.15–4.75), whereas K_A factors remained unaffected by processing (5.63 ± 0.03). Similarly, K_p factors of mealworm meals varied with processing (4.53–5.50), while K_A values remained constant (5.72 ± 0.02). In both crickets and mealworms killed by freezing, the drying method strongly impacted $N_{NP,NC}$ fractions as well as meal colour, with $N_{NP,NC}$ accounting for 19% and 37% of N_{total} in oven-dried crickets and mealworms, respectively. In contrast, blanched insects had similar $N_{NP,NC}$ concentrations (8–10% of N_{total}). These results indicate that non-protein nitrogenous compounds were generated through proteolysis and enzymatic browning during drying. Protein contents calculated using N_P and K_A ranged from 33–37% for cricket meals and 19–42% for mealworm meals, compared to 49–50% for both insects when using N_{total} and a K_p factor of 4.76. Thermal processing significantly affects the K_p value of insect meals, limiting its reliability. Therefore, protein quantification based on protein-bound nitrogen and K_A factors of 5.61 (*A. domesticus*) and 5.69 (*T. molitor*) provides a more suitable approach for determining protein content in insect ingredients.

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Effect of storage temperature and duration on lipid oxidation of black soldier fly larvae meal

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Black soldier fly larvae meal (BSFLM) is considered a promising alternative protein source and a valuable lipid source for poultry. However, the elevated lipid fraction may increase susceptibility to oxidative deterioration during storage and under environmental temperatures typical of poultry housing conditions. This study evaluated the effects of storage temperature and storage duration on lipid stability of BSFLM. Samples were stored at +4 °C, 25 °C, and 35 °C for 14 and 32 days. Free acidity (%) and thiobarbituric acid reactive substances (TBARS, mg MDA/kg) were determined. Data were analysed using two-way ANOVA to evaluate the main effects of temperature and storage duration, as well as their interaction. Free acidity was significantly affected by both temperature (P = 0.022) and storage duration (P < 0.001), while their interaction was not significant (P = 0.163). Mean free acidity increased from 11.41% on day 14 to 12.69% on day 32. TBARS values were significantly influenced by storage duration (P < 0.001) and showed a strong temperature × duration interaction (P < 0.001). The highest TBARS value was observed at 25 °C after 32 days (4.93 mg MDA/kg), whereas the lowest value occurred at 25 °C after 14 days (3.67 mg MDA/kg). Temperature-related differences were limited at shorter storage periods but became more pronounced with prolonged storage. These findings indicate that storage duration is a key driver of lipid oxidation in BSFLM, while temperature effects emerge mainly during extended storage. Appropriate storage strategies, particularly under poultry house temperature conditions, are therefore essential to preserve oxidative stability and nutritional quality of insect-based feed ingredients.

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Functional bioactivities of chitin-rich fractions from black soldier fly larvae (*Hermetia illucens*)

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Edible insects are increasingly recognized as sustainable sources of protein and nutrients for food and feed systems; however, research supporting their functional health benefits remains limited. This study evaluated the antiglycation and digestive enzyme inhibitory activities of black soldier fly larvae (BSFL; *Hermetia illucens*) extracts to explore their potential as functional bioactive ingredients. Protein, fat, and chitin-rich fractions were obtained from BSFL using bioassay-guided fractionation. Defatted samples were further processed to generate chitin-rich fractions using chemical extraction and microwave-assisted extraction techniques. All extracts were assessed *in vitro* for α-amylase and α-glucosidase inhibitory activities using chromogenic substrates, while antiglycation activity was evaluated using an advanced glycation end-product–bovine serum albumin (AGE-BSA) model system. The inhibition of protein-bound fluorescent AGEs varied significantly among BSFL fractions, with IC_{50} values ranging from 125.19 ± 13.35 to 464.74 ± 56.84 μg/ml. Chitin–protein mixtures and mineral-containing chitin-rich fractions exhibited significantly stronger antiglycation activity than mineral-free chitin fractions. Multivariate analysis confirmed fraction type (% variance = 68.71–80.61%, $p < 0.0001$) as the primary driver of antiglycation activity, whereas substrate type (% variance = 6.041%, $p = 0.0567$) had only a marginal effect. Principal Component Analysis showed the following relations (i) PC1 (74.5%) and PC2 (8.9%) dimensions covering the largest portion of variance with a total of 83.4% and (ii) PC1 (74.5%) and PC3 (5.9%) accounted for 80.4% of the variance. The protein/chitin extract showed the highest α-amylase inhibitory activity (IC_{50} = 3780.00 ± 58.59 μg/ml), while α-glucosidase inhibition was limited across all extracts. Notably, chemically extracted chitin-rich fractions (IC_{50} = 239.40 ± 19.45 μg/ml, $p < 0.05$) demonstrated superior antiglycation activity compared with microwave-assisted extracts (1065.70 ± 31.64 μg/ml) and the positive control aminoguanidine (IC_{50} = 558.40 ± 23.70 μg/ml). Overall, *H. illucens* chitin-rich fractions display promising antiglycation and α-amylase inhibitory activities, supporting their potential application as functional ingredients in sustainable food and feed systems.

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From rearing and processing to gastrointestinal digestion: oxidative and glycative reactions in black soldier fly larvae

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The use of insect protein in animal and human nutrition is increasing, driven by its sustainability potential and its value as an alternative protein source. However, processing-induced oxidative reactions and their evolution during gastrointestinal digestion remain poorly understood, despite their potential impact on protein digestibility and the formation of bioactive oxidation products. This study investigated how rearing substrate and processing conditions interact to shape the oxidative stability of black soldier fly (BSF) larvae. A 4 × 2 × 2 factorial design was applied, combining different rearing substrates (tofu, brewery and rice by-products, and chicken feed), killing methods (blanching vs. blast-freezing), and drying techniques (oven- vs. freeze-drying). Fatty acid and mineral profiles of both feed and larvae were determined. Oxidative stability was assessed before and after *in vitro* gastrointestinal digestion through targeted markers of lipid oxidation (propanal, hexanal, 4-hydroxy-2-nonenal) and Maillard reaction products (MRPs). Rearing substrate strongly modulated the fatty acid profile and mineral contents of larvae, influencing their susceptibility to oxidation. However, processing conditions were the dominant determinants of oxidative reactions. Blast-freezing generally limited lipid oxidation compared to blanching, whereas specific combinations of blast-freezing and drying promoted MRP formation. Higher MRP levels were associated with lower lipid oxidation, suggesting that processing-induced MRPs may contribute to lipid oxidative stabilization during digestion. Overall, these findings indicate that while rearing influences the compositional predisposition of insect ingredients, processing strategies largely determine their chemical reactivity during gastrointestinal digestion. Strategic optimisation of rearing and processing conditions offers opportunities to improve the nutritional and oxidative quality of insect-based protein ingredients.

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Bacterial microbiota analysis of *Hermetia illucens* larvae feed with meat-based former foodstuffs

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Edible insects are recognised as a sustainable source of animal protein for food and feed, and several formulations have been authorised within the European Union. Among edible insects, black soldier fly (*Hermetia illucens*) larvae (BSFL) can efficiently convert organic waste and former foodstuffs (FFs) into protein-rich biomass, representing sustainable and economically viable feed and food. However, EU food and feed legislation currently prohibits the use of meat and fish as components of rearing substrates in insect farming. Indeed, safety concerns persist, and additional data are needed to evaluate the risks associated with the use of meat and fish based FFs as BSFL substrates. In this study, the bacterial microbiota of BSFL reared on substrates containing raw and processed meat based FFs was investigated, together with the effects of post-harvest treatments including fasting, rinsing with distilled water, and washing with 70% ethanol (EtOH) solution. Three substrates were tested: a 100% plant-based control (C), and two substrates containing 25% of FFs raw meat (R) or 25% FFs processed meat (P). BSFL were sampled at the end of the rearing period as untreated larvae, after 24 h fasting, rinsing, and EtOH washing. Bacterial DNA was extracted and analysed using 16S rRNA metabarcoding. The bacterial community was dominated by *Pseudomonas* spp., *Providencia* spp., *Myroides* and *Sphingobacteriaceae*. Major foodborne pathogens, including *Listeria* spp., *Staphylococcus* spp., *Salmonella* spp. and *Campylobacter* spp., were not detected. As expected, both alpha and beta diversity analysis revealed that substrate type significantly affected the BSFL bacterial microbiota. Specifically, Shannon and Simpson diversity indices were significantly higher in BSFL reared on C and R substrates, suggesting that the use of processed FFs as part of the rearing substrate may reduce the BSFL bacterial microbiota biodiversity and potentially the level of microbial contamination. On the contrary. fasting, rinsing, and EtOH washing had limited impact on the bacterial microbiota. These results support the potential use of BSFL in circular economy strategies. However, further investigations are needed before meat based FFs can be considered safe substrates for food or feed production.

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Valorising unsold supermarket products through black soldier fly larvae rearing

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Recently, the European Parliament adopted new measures to prevent and reduce food waste across the EU by 2030. To date, several former foodstuff products could be used as feed ingredients if they do not contain meat or fish. The aim of this research was to test the effect of substrates made with different unsold supermarket products on the growth performance and chemical composition of BSF larvae. Products from a local supermarket removed from sale for nearing expiration date or quality issues were collected. Three substrates were formulated: (P), composed of a mix of fruits and vegetables; (C), with 75% P and 25% of a cheese mix (on a wet basis, WB); (Y), with 75% P and 25% yoghurt mix (on a WB). Growth rate (GR), feed conversion ratio (FCR), efficiency of conversion of ingested feed (ECI), substrate reduction (SR) and waste reduction index (WRI) were calculated. Furthermore, the proximate composition, fatty acid profile, antioxidants and mineral concentrations were analysed in substrates and larvae. The results showed that C larvae had the highest average larval weight. Accordingly, the C larvae had the highest GR, SR, WRI and the lowest FCR in comparison to P and Y larvae. The CP% (DM) content does not differ significantly among P, C, and Y larvae. However, the highest ether extract content (EE%, DM) was in the C substrate, while the highest EE% content was in Y larvae, consistent with the presence of soluble sugars in the yoghurt. The fatty acid profile of larvae (%FA on total FA) changed in the total amount of saturated and monounsaturated FA, resulting in higher concentrations in C and Y than in P larvae. The levels of tocopherols and carotenoids (μg/g) in larvae varied with the different proportions of plant-based substrate. P larvae resulted in the highest concentrations of Ca, K, P, and Mg (mg/kg DM). These results support the growing body of evidence on the significant role of BSF larvae in reducing food losses and increasing the sustainability of the agrifood system. Acknowledgement: The work done at IRTA is financed by the European Union's European Regional Development Fund, within the framework of the Catalonia FEDER Programme 2021-2027 and the STEP initiative.

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Sublethal effects of cyromazine on life-history characters and reproductive performances of *Hermetia illucens*

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The circular economy is increasingly emphasized in food and feed systems due to the 1.3 billion tonnes of agricultural by-products generated annually. Black soldier fly (*Hermetia illucens*) farming represents a promising bioconversion strategy; however, widespread pesticide use and presence in residue for larval substrates, may compromise immature and adult performances, while transferring the active compounds through the food chain. Cyromazine, an insect growth regulator, was assessed on larval weights, growth rates, feed conversion ratio frass corrected (FCR frass corrected), efficiency on conversion of ingested (ECI) and digested (ECD) feed and reproductive performances of *Hermetia illucens*. Larvae were fed, in triplicates, 1 kg of Gainesville diet (i.e., standard feedstock) at 70% moisture (control) or four Gainesville diets contaminated with cyromazine (0.0625, 0.125, 0.25, 0.5 ppm). Ten largest larvae were weighed daily and sieved three days after reaching a maximal stable weight, then transferred to plastic boxes for emergence. Sixty adults (40 males, 20 females) were sexed and placed in a cage for each replicate, and mating and oviposition events were recorded daily for 12 h. Only the highest treatment (0.5 ppm) significantly affected larval weights, whereas no differences were observed between the other treatments and the control (0.170 g ± 0.004, 0.166 ± 0.006, 0.162 ± 0.000, 0.162 ± 0.003, and 0.136 ± 0.004, from control to 0.5 ppm, $P < 0.05$). Similarly, bioconversion indexes FCR frass corrected, ECI and ECD were not negatively impacted by the presence of the contaminant, except for 0.5 ppm (2.81 ± 0.17, 2.95 ± 0.08, 3.00 ± 0.17, 3.04 ± 0.15, 5.18 ± 0.71 for FCR frass corrected, 0.36 ± 0.02, 0.34 ± 0.01, 0.33 ± 0.02, 0.33 ± 0.02, 0.20 ± 0.03 for ECI and 0.39 ± 0.02, 0.37 ± 0.01, 0.37 ± 0.02, 0.36 ± 0.02, 0.23 ± 0.03 for ECD, from control to 0.5 ppm, $P < 0.05$). Adult reproductive performance differed among treatments. Cyromazine affected negatively the number of mating events. From 0.25 ppm, cyromazine presence increased oviposition events; however, the amounts of eggs laid per oviposition event were significantly lower – by up to 40% – than in 0.0625 ppm and the control. Total egg production did not differ significantly among treatments. These results show that sublethal cyromazine exposure may negatively affect growth performances, conversion and efficiency indexes, as well as the reproduction performances of the adults. Results highlight the need of close monitoring for the presence of chemical and pesticides residues in agricultural by-products when used as substrates in the insects farming sector.

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Effects of zinc on life-history traits including heat resistance of *Hermetia illucens* larvae

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Zinc is an essential trace element involved in physiological processes in all living organisms, including heat resistance; however, excessive levels can be toxic. Heavy metal contamination in byproducts-derived substrates is a major concern in insect farming. Larvae of the black soldier fly (*Hermetia illucens*) are widely used for bioconversion of low-quality substrates, yet heavy metals may impair larval performance, highlighting the need for careful monitoring of substrate contamination. In this study, approx. 1000 7-day-old *Hermetia illucens* larvae were fed diets (1 kg) containing double (D, 6 mg) and triple (T, 9 mg) the zinc concentration normally present in their standard basal diet Gainesville (G, 3 mg), which served as the control. Treatments were performed in triplicate. Larvae were reared until reaching 19 days of age. To evaluate whether increased zinc intake affected larval resistance to elevated temperatures, heat resistance was assessed at completion of 75% of development of both larval stage and prepupal stage, calculated based on accumulated degree days (ADD). Accordingly, ten larvae per treatment were weighted and subjected to the experimental procedure at 11 and 19 days of age. Larvae were placed in glass vials and immerged in a water bath (reverse osmosis water). Water temperature (27 °C) was gradually increased, and heat knockdown was recorded when each larva ceased movement. Conversion and efficiency indexes (Feed Conversion Ratio frass corrected, Efficiency of Conversion of Ingested Food ECI, and Efficiency of Conversion of Digested Food ECD) were assessed. Results show that growth performances were not impacted by the zinc concentration of the diets, as average final weights did not significantly differ between treatments (G: 56.47 g; D: 61.73 g; T: 74.20 g;). Similarly, performance indexes did not show statistical differences, therefore zinc at the levels examined did not exert toxic effects (G: 5.818, D: 5.555, T: 4.663 for FCR frass corrected; G: 0.174, D: 0.182, T: 0.214 for ECI; G: 0.195, D: 0.202, T: 0.233 for ECD). Regarding the heat treatments, average knockdown temperatures were similar between treatments both at 11 (G: 50.5 °C; D: 50.3 °C; T: 50.1 °C) and at 19 days of age (G: 50.9 °C; D: 50.7 °C; T: 50.3 °C). These data indicate that zinc concentrations up to three times the baseline level present in a standard diet do not exert toxic effects on *Hermetia illucens* larvae and, at the same time, did not improve larval resistance to heat stress.

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Transfer and accumulation of arsenic, cadmium and lead in farmed insects from endogenous substrate contaminants

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Uptake and accumulation of undesirable trace elements is a complex process governed by both physico-chemical and biological variables. Many studies on trace element contamination in farmed insects rely on spiked substrates, producing unrealistically high contaminant levels and potentially overestimating bioavailability. This study examined transfer of endogenous arsenic (As), cadmium (Cd) and lead (Pb) under lab and production-scale rearing conditions using 27 practical feed substrates and five insect species: *Hermetia illucens*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus* and *Locusta migratoria*. Element concentrations were measured via ICP-MS, and bioaccumulation factors (BAF) determined on a dry-matter (DM) basis. Substrate concentrations varied widely, with maxima of 1.15 mg As/kg DM, 1.43 mg Cd/kg DM and 13.60 mg Pb/kg DM. In contrast, insects showed narrower ranges, with highest observed levels of 0.386 mg As/kg DM, 1.066 mg Cd/kg DM and 2.085 mg Pb/kg DM. Bioaccumulation of As and Pb was not observed in any species (all BAF<1). Cd bioaccumulation occurred in *H. illucens* (mean BAF 3.74) and *T. molitor* (mean BAF 3.07), whereas *L. migratoria* showed low mean BAF values across all elements (As 0.17, Cd 0.36, Pb 0.18). All insects were below EU feed ingredient limits for As, Cd, and Pb. In the context of novel food legislation, *A. diaperinus* and *A. domesticus* met all regulatory thresholds, while *L. migratoria* exceeded Cd and Pb limits when reared on highly contaminated grass. One *T. molitor* sample slightly exceeded the Cd limit for frozen larvae (0.051 mg/kg WM vs. 0.050 mg/kg WM). Importantly, Pb levels in all *T. molitor* samples were below the analytical LOD of this study (0.023 mg/kg WM), preventing confirmation of compliance with the extremely low permitted Pb levels (0.01–0.02 mg/kg). This study provides the most comprehensive evaluation to date of endogenous trace element transfer in farmed insects under realistic production conditions. Results confirm Cd bioaccumulation in *H. illucens* and *T. molitor*, reveal strong element exclusion in *L. migratoria*, demonstrate broad compliance with feed safety standards, and highlight regulatory challenges when legal limits fall below achievable analytical sensitivity.

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Influence of agro-industrial by-product diets on the nutritional values of *Tenebrio molitor*

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Intensive livestock and aquaculture systems require high-quality feed that is nutritionally balanced. The decrease in wild fish protein has led to demands within the feed supply chain for new protein sources to fulfil the growing demand. In this context, edible insects such as the yellow mealworm (*Tenebrio molitor*) are the most promising alternative protein source. This study evaluated the nutritional profile of yellow mealworm larvae reared in a laboratory on eight different agro-industrial by-products: wheat middlings, durum wheat bran, rice bran, hemp cake, thistle cake, dried brewer's spent grains, dried tomato pomace, and dried distillery grape marc. The quantitative and qualitative impacts of the rearing substrates on the larvae were compared. The results showed that the larvae adapted well to different substrates with different nutritional compositions, including the fibrous fraction. However, substrates affect larval macronutrient composition. Hemp cake stood out for its superior nutritional value, as reflected by its high protein content and moderate NDF levels. Conversely, imbalanced lipid or carbohydrate content in the substrate (rice bran) and the presence of potential antinutritional compounds (thistle cake) appeared to negatively affect *Tenebrio molitor*. The larvae of *T. molitor* are a stable protein source but variable for lipids and moisture. The substrates recommended are hemp cake, wheat bran, and wheat middling. Substrates that should be avoided include RB, TC, and DGM. Analysis performed on residual feed and insect frass supported the selective feeding behaviour and high plasticity of mealworm larvae and their potential to adapt to different substrates. Indeed, larvae try to maximise the intake of sugars, fats and amino acids while minimising the ingestion of fibre.

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The efficient degradation mechanisms of aflatoxin B1 by *Hermetia illucens* combined with gut microbe *S. geniculata* ZJA10

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Aflatoxin contamination is a widespread and persistent problem in agriculture sector, posing serious risks to food safety, public health, economy and international trade. Consequently, the development of environmentally friendly and efficient approaches for aflatoxin removal has become a global priority highlighting microbial biodegradation. Recently, Black soldier fly larvae (BSFL; *Hermetia illucens*) has gained attention for its remarkable ability to bioconvert organic waste and bioremediation in general. This ability has largely attributed to its diverse and active gut microbiota. In this study, natural consortium was derived from BSFL gut and evaluated their ability to degrade AFB1 over 16 days. It was revealed that the AFB1 concentration decreased to 50% then 99% in the day 4 and 8 respectively and reached complete degradation on the sixteenth day. Metagenomic analysis was used to understand microbial community composition during these days. It was revealed that Pseudomonadata taxa was the predominant taxa participating in AFB1 biodegradation in these enriched natural consortia. Additionally, there are significant shifts in microbial diversity composition which reveal the most adapted genera and species. Subsequently, isolation of efficient AFB1 degrading bacterial strains was performed and inoculated sterile BSFL with them. The inoculated sterile BSFL showed a remarkable decline in AFB1 concentration particularly the BSFL inoculated by *Stenotrophomonas geneiculata* ZJA10. So ZJA 10 strain was selected as the most efficient strain for whole genome sequencing to investigate the genetic determinants behind AFB1 degradation. This study integrates multi-omics and culturomics approaches to elucidate the role of BSFL gut microbiota in AFB1 biodegradation, highlighting their potential as valuable bioresources for the remediation of aflatoxin contamination.

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Nutritional quality and oxidative status of house crickets (*A. domesticus*) in iron-enriched rearing conditions

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Dietary iron deficiency remains a public health challenge in low-income countries. Insects are a source of highly bioavailable iron; iron enrichment of *A. domesticus* might mitigate the low iron bioavailability. Iron is an essential micronutrient for metabolic processes, but it has toxic effects when in excess. This study investigated the effects of dietary iron supplementation on rearing performance and oxidative status of house crickets (*Acheta domesticus*). Neonate crickets (20 days old) were reared under controlled conditions (31 ± 1 °C temperature, 50 ± 5% RH, 12:12 (L:D) photoperiod), and fed with commercial chicken feed supplemented with increasing $FeSO_4$ concentrations as the iron source (1, 5, 10, 25 and 100 mmol Fe/kg diet). Five replicates/treatment were prepared. The first adult emerged after 21 days in every treatment. Growth parameters (survival, weight gain, and rearing efficiency) and lipid peroxidation (TBARs) were evaluated. The survival was significantly higher in the 1 (91.2 ± 3.43%), 5 (96.42 ± 1.90%) and 10 (91.65 ± 4.31%) mmol Fe groups compared to the CTR (88.46 ± 1.22%), while it was decreased in the 25 (88 ± 2.71%) and 100 (86.88 ± 3.15%) mmol Fe groups. The total feed intake was higher in the 1-25 mmol Fe groups (20.09 ± 0.64, 17.24 ± 0.76, 17.07 ± 1.34, 15.82 ± 0.79 g) compared to the CTR (15.78 ± 0.73 g) and lower in the 100 mmol Fe treatment (12.59 ± 0.43 g). The Feed Conversion Ratio (FCR) was lower in the 100 mmol Fe group (0.98 ± 0.04) compared to all the other treatments, indicating altered feeding behavior and metabolic stress. Oxidative stress was higher in the 100 mmol Fe group (199 ± 29.02 μg MDA/kg) compared to the CTR (120 ± 6.23 μg MDA/kg). No significant differences were observed between CTR and the other groups (values ranged between 135.00 ± 29.64 and 136.00 ± 19.86 μg MDA/kg). The results highlight that optimised dietary iron enrichment improves *A. domesticus* growth while limiting lipid peroxidation. In conclusion, iron supplementation of 5-10 mmol Fe/kg diet is recommended for optimising cricket rearing, thereby guaranteeing low oxidative damage and ensuring the adequate nutritional quality of edible crickets.

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Structural and protein quality characterisation of a black soldier fly larvae protein concentrate by ASIP

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Black soldier fly larvae (BSFL, *Hermetia illucens*) are gaining interest as a sustainable protein source for food and feed due to their high protein content and low environmental footprint. BSFL-derived ingredients are therefore investigated as alternatives to conventional protein sources. In this study, the nutritional composition of BSFL flour and the protein quality, structural characteristics and antinutritional profile of a protein concentrate obtained by alkaline solubilisation coupled to isoelectric precipitation (ASIP) were characterised to evaluate its potential as a functional protein ingredient. BSFL flour exhibited high protein (38.7%), fat (27.9%) and ash (10.5%), indicative of substantial mineral density. The mineral profile showed high levels of essential minerals, including calcium (37.13 g/kg DM), magnesium (4.82 g/kg DM), and potassium (14.95 g/kg DM), and trace elements such as iron (282.13 mg/kg DM), zinc (174.67 mg/kg DM) and copper (19.69 mg/kg DM). ASIP yielded a protein concentrate containing 69.6% protein, with extraction yield of 25.2% and protein recovery of 14%. Amino acid analysis demonstrated all essential amino acids; histidine was limiting (AAS 62.67%) while all others exceeded the FAO (2013) reference pattern. The protein concentrate showed high *in vitro* protein digestibility (IVPD = 80.39%) and IVPDCAAS of 50.38%. Total polyphenols were detected in both flour (125.22 mg/100 g) and protein concentrate (102.50 mg/100 g), whereas tannins, saponins, phytic acid, and trypsin inhibitor activity were absent or negligible. FTIR spectroscopy of the protein concentrate indicated α-helix (17.89%), β-turn (11.12%), β-sheet (30.25%) and random coil (40.74%), while surface hydrophobicity (Ho) was 3177.7, indicating pronounced exposure of hydrophobic groups that may influence functional behaviour. Overall, these findings demonstrate that BSFL provides high nutritional value, favourable amino acid profile and low anti-nutritional factors, supporting its potential as a sustainable protein ingredient, though further optimisation of protein recovery is needed to improve yield and process efficiency.

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Acheta domesticus or *Tenebrio molitor*? Species-dependent effects of insect powders on wheat bread quality

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Despite the growing interest in insect-enriched bakery products, direct comparative studies assessing how the addition of different insect species affects bread quality remain limited. This study aimed at comparing the effects of adding *Acheta domesticus* or *Tenebrio molitor* powders as partial replacements for wheat flour on bread colour, baking loss, crumb hardness, and sensory acceptance. Wheat flour was partially replaced with *Acheta domesticus* or *Tenebrio molitor* powder at levels of 5, 10, 15 and 20%. The breads were prepared using ingredients, including oil, salt, sugar, and dried yeast. Colour parameters (L^*, a^*, b^*), crumb hardness (penetration test), baking loss, five sensory attributes (flavour, aroma, hardness, chewiness and gumminess), and overall acceptability were evaluated using a 9-point hedonic scale. Statistical analysis was performed using two-way ANOVA. Both insect species and inclusion level significantly affected most quality parameters. Increasing insect content decreased lightness (L^*), with a more pronounced darkening effect observed in *Acheta domesticus*. Colour parameters a^* and b^* differed significantly between species, indicating a distinct influence of insect raw material on bread colour. Crumb hardness depended on both species and inclusion level, though it was less pronounced than colour and sensory changes. Baking loss varied among formulations, without a clear species-dependent trend. Sensory evaluation revealed significant differences between insect species. Breads containing *Acheta domesticus* received higher scores for flavour and overall acceptability than those with *Tenebrio molitor*. Increasing insect addition led to a gradual decrease in sensory acceptance regardless of species. *Acheta domesticus* showed a more favourable impact on sensory attributes, which may be related to differences in chemical composition and interactions with the gluten matrix. In contrast, *Tenebrio molitor* had a greater effect on colour and consumer acceptance at higher inclusion levels. Conclusions *Acheta domesticus* demonstrates greater application potential than *Tenebrio molitor*, particularly at moderate inclusion levels (5–10%), highlighting the importance of species-oriented formulation strategies in the development of insect-based bakery products.

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Lipid profile modulation in *Hermetia illucens* larvae fed olive pomace and brewer's spent grain

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The valorisation of agro-industrial by-products via *Hermetia illucens* (BSF) offers a sustainable route for nutrient recovery. This study assessed how substrates with distinct chemical profiles – olive pomace (OP, lipid-rich) and brewer's spent grain (BSG, carbohydrate/protein-rich) – influence the lipid composition and fatty acid bioconversion in BSF larvae. Six-day-old larvae were reared (30 °C, 60% RH) on OP or BSG until the prepupal stage. Substrates, larval biomass and frass were analysed for proximate composition and fatty acid profiles using gas chromatography (FAMEs). Substrate composition directly modulated larval macronutrients. Larvae reared on OP (15% lipid, around 57% oleic acid) accumulated high lipid levels (57.5% dry weight) with an oleic acid-rich profile (39%), indicating bioaccumulation. Conversely, larvae fed BSG (6.6% lipid, rich in carbohydrates) showed a leaner profile (36% lipid) but demonstrated significant *de novo* biosynthesis. Notably, lauric acid (C12:0) increased significantly in BSG-fed larvae, comprising >62% of total oil compared to approx. 10% in the substrate. OP-fed larvae also enriched lauric acid to around 16% (vs approx. 3% in substrate). BSF larvae exhibit metabolic plasticity, functioning as active lipid bioreactors rather than simple accumulators. While lipid-rich substrates promote fat accumulation, carbohydrate-rich sources like BSG stimulate the synthesis of high-value medium-chain fatty acids (lauric acid), tailoring the oil for specific animal nutrition applications.

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Experimental model for evaluating rearing conditions and bio-conversion by BSF

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The MicroDEMi experimental model was designed to evaluate rearing conditions, feeding diets, and genetics of black soldier fly (BSF) using controlled experimental units. Experimental conditions, including ambient parameters and larval rearing duration, are predefined to align with industrial realities. The model allows for twenty-five replicates per experimental condition and up to 4 conditions + control per trial, enabling robust statistical evaluation. This framework provides a valuable tool for assessing raw materials, genetic performance and optimising growth time in BSF farming.

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Water spinach as a green feed supplement: impacts on survival and feed utilization in two-spotted crickets

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Insect farming may support more sustainable protein systems by using locally available feed resources. Water spinach (*Ipomoea aquatica*) is widely available in Vietnam, but experimental evidence for its use in cricket rearing is limited. We tested whether adding water spinach leaves to commercial broiler feed affected growth performance and survival of the two-spotted cricket (*Gryllus bimaculatus*) under captive conditions. Two diets were compared: (T1) commercial broiler feed (control) and (T2) broiler feed plus fresh water spinach leaves. Each diet had 8 replicates; each replicate was a 15-litre plastic box stocked with 200 one-day-old crickets (n = 3200). After one week of adaptation, data were recorded for four weeks. Outcomes included average daily gain (ADG), feed conversion ratio (FCR), and survival at different stages. Data were analysed using a general linear model ($P < 0.05$). ADG was similar between diets (T2: 41.85 vs T1: 39.08 mg/individual/day; $P = 0.71$). FCR tended to be higher in T2 than T1 (2.08 vs 1.42; $P = 0.06$). Survival differed by stage: in the first two weeks, survival was lower in T2 than T1 (47.88% vs 60.00%; $P < 0.01$), while harvest survival was numerically higher in T2 (73.22% vs 58.36%; $P = 0.20$). Water spinach can be used as a low-cost local green supplement for *G. bimaculatus*, but it involves trade-offs. Supplementation did not increase growth significantly and tended to increase FCR, and early survival was lower. Future work should optimise supplementation strategy and early-stage management to improve overall performance.

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"Micro" nutrients, major impacts: effects on growth and nutritional value of *Tenebrio molitor* larvae

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Global food demand continues to rise, while micronutrient deficiencies remain widespread. Micronutrients occur naturally in soil and plants and are essential for human and animal health. Insects, such as *Tenebrio molitor*, can efficiently convert various substrates into high-quality feed and food products and their nutritional value can be influenced by the feeding substrate. This study evaluated the effects of dietary supplementation with four micronutrients (Mn, Zn, Ca, P) at three inclusion levels on the growth and nutritional composition of *T. molitor* larvae. Manganese was added at 10, 20 and 200 ppm, zinc at 15, 30 and 300 ppm, and calcium and phosphorus at 0.5, 1 and 2%. All micronutrients were incorporated into wheat bran, which served as both the experimental base and the control diet. Larval growth and survival were monitored weekly and the experiment concluded with the appearance of the first pupa. Feed utilisation parameters, including Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR), were calculated and chemical analyses were performed to determine larval mineral composition. Significant dietary effects were observed for most parameters, while development time was less affected. Diets supplemented with manganese consistently produced the highest larval weights (107–113 mg), survival rates of approx. 97%, and improved feed efficiency. By contrast, zinc-supplemented diets, particularly at medium and high levels, resulted in lower larval growth (98–103 mg) and survival of 83–88%. Calcium- and phosphorus-enriched diets supported intermediate to high growth and survival, with larval weights of 103–106 mg and 106–109 mg, respectively, and survival up to approx. 90%. Zinc at 30 ppm yielded the largest FCR (approx. 2), while the maximum phosphorus inclusion led to the lowest SGR (7.72%). Mineral analyses revealed similar concentration ranges across treatments, with some variability. Overall, these results demonstrate that targeted micronutrient supplementation can enhance *T. molitor* growth, survival, and feed efficiency, providing valuable insights for the optimisation of insect-based feed and food production.