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Foreword

This year marks the 10th anniversary since the inaugural "Insects to Feed the World" conference held in Wageningen, the Netherlands, and each meeting brings together additional participants from around the world. Since the first meeting, IFW conferences have been held in Wuhan, China in 2016, as well as Quebec, Canada, which was held in a virtual event in 2020, and an in-person event in 2022, attended as well by online participants.

The 5th edition of Insects to Feed the World from June 18th to June 22nd 2024 proudly takes place in Singapore, and is hosted by the Asian Feed and Food Insect Association (AFFIA). IFW 2024 strives to create an inclusive environment to join diverse participants from academia, industry, government, and cultural backgrounds spanning the globe. To advance an agenda for the inclusion of edible insects and insect agriculture in tomorrow's sustainable food systems, an integrated approach is required to connect the multi-faceted elements necessary for shared knowledge, innovation, and effective development strategies. With a hyper-diverse and highly multicultural population, and a known centre for food worldwide, Singapore is an excellent venue for this dialogue.

Singapore is keenly aware of the need for food security, and alongside food, insects can also play a key role in supporting other food systems such as aquaculture, and leading research in the use of insects in tropical aquaculture has been highlighted in a dedicated keynote panel on this topic, showcasing researchers from across East and Southeast Asia. Additionally, a technical tour of several of Singapore's aquaculture facilities working on insects as aquafeed ingredients, as well as visits to research labs, teaching facilities, start-ups, and community outreach initiatives highlighted several of the actors necessary for a holistic integration of these sustainable elements of insect agriculture.

The conference also held keynote panels from investors and leading insect researchers to provide key insights from these sectors, and keynote speakers drawn from broad backgrounds and identities from across the globe led discussions on broad topics ranging from insect breeding, production, and animal well-being. Key presentations from governmental and religious organizations also contributed important considerations to highlight the need for regulatory and cultural acceptance of these novel foods.

Importantly, the third Global Roundtable for Insect Agriculture (GRIA) took place, which facilitated dialogue with international industrial associations, and the founding of the Academic Society for Insects as Food and Feed (ASIFF) was announced, cementing a society dedicated to advancing international research efforts for the implementation of insects as food and feed.

Thus, the 5th edition of Insects to Feed the World has striven to establish an inclusive environment peopled by diverse voices and identities to ensure a collective dialogue that enables the continued development and innovation in the use of insects as food, feed, and other sustainable approaches that prepare the world for a sustainable future. We look forward to the next instalment of IFW and hope you will join us there to see what that future holds!

Eric C. Peterson and Christian Hermansen
Program Co-chairs, on behalf of the Local Organizing Committee for Insects to Feed the World Singapore 2024

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KEYNOTES

Insect sector current developments and future prospects

K. Aarts

Protix, Dongen, the Netherlands

KEYNOTES

Insect farming for a circular bio-economy in controlled environment agriculture

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Controlled environment agriculture (CEA) involves the production of plants – including algae – fungi, insects, shrimps and fish in almost closed and regulated systems to enable the decoupling of production from seasons, climate and location. CEA aims higher productivity with consistently high product quality, reduction of environmental and health stresses (nutrient leaching, water consumption, land use) as well as application of pesticides, herbicides and antibiotics. In this context, insect farming promotes the coupling of different CEA production systems for a resilient and resource-optimized agriculture aiming zero waste in a circular bioeconomy (Circonomy©) even in urban landscapes such as Singapore. Farmed insects such as the larvae of the black soldier fly Hermetia illucens are considered as a missing link for the circular bio-economy in CEA because they can mediate the industrial bioconversion of agricultural or industrial side streams into feed for aquaculture and livestock. In turn, the leftovers of farmed insects, the so-called frass (excrements, chitinous exuvia of the molting larvae and feed leftovers), represents a valuable biofertilizer, which can replace chemical fertilizer in agriculture and vertical farming. The ability of H. illucens larvae to utilize almost all organic substrates, even liquid manure, as a diet and to produce a potent a biofertilizer has been attributed to their beneficial microbes in the gut, which can also mediate the detoxification of plant-derived secondary metabolites. The presentation highlights the development of a pathogen detection system to prevent in industrial insect farming both the outbreak of diseases and the contamination with food-born pathogens such as Listeria- or Salmonella bacteria. The avenues for revenues of industrial insect farming can be expanded beyond the production of food, feed and bio-fertilizer to encompass the development of higher-added value products from the generated protein, lipid and chitin fractions. Taken together, insect farming can integrate several forms of CEA to create a sustainable and circular bio-economy in the food and feed industry.

KEYNOTES

Insect reproductive evolution: from biological diversification to sustainable solutions

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Insects represent much of terrestrial biodiversity and play crucial roles in the health and survival of our ecosystems. They are pollinators, nutrient cyclers, and the main dietary source for numerous other invertebrate and vertebrate species. Recent studies have highlighted the growing threat to insect numbers globally and there is an urgent need to understand species diversity and distribution, especially in tropics which are often hotspots for biodiversity. Investigating the reproduction of insects can shed light on both proximate mechanisms and ultimate patterns that are behind biological diversification. Reproductive strategies in this lineage can vary from the ancestral strategies of direct development and oviparity to the more complex forms involving viviparity, sociality, and even parental care. This array of mating systems have been shaped by both natural selection (e.g. resource availability, predation pressure, etc.) as well as sexual selection (e.g. mate acquisition, non-random mate choice, postcopulatory competition, etc.). This presentation will delve into the fundamental evolutionary processes, highlighting the incredible adaptability of insects in response to diverse ecological challenges as well as to potential applications in sustainability. Using empirical evidence from ecosystem service providers such as dung beetles, to medically relevant vectors such as mosquitoes and recycling powerhouses such as black soldier flies, this presentation will emphasize the continued need for investigating reproductive evolution in Nature's tiny creatures.

KEYNOTES

Insects for food & feed security

C.M. Tanga

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KEYNOTES

Welfare science: a pillar of values-driven agriculture

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The growing insects as food and feed (IAFF) industry has tremendous potential to improve food security and support a more sustainable agricultural system. The insights of welfare science in other animal agricultural industries have supported economic productivity, created product differentiation opportunities, and helped maintain their social license to operate. These same benefits may also be provided to the IAFF industry by the growing field of insect welfare science. Early efforts in insect welfare science have identified important welfare challenges in industrial-scale insect rearing that, if addressed, could enhance production, lower costs, and ultimately increase profits. Using the black soldier fly as a case study, I review factors that may impact the welfare of black soldier fly larvae and adults. I provide examples from my ongoing research program of how empirical research can offer pathways to solutions for welfare challenges in the industry. First, I discuss data on humane slaughter and depopulation of black soldier fly larvae. Next, I discuss data on adult black soldier fly nutrition. While these datasets offer promising future directions for the IAFF industry, I also discuss why both demonstrate the necessity of developing innovative collaborations between researchers and producers to find welfare solutions that ultimately serve the industry's needs. I end by discussing open research questions for improving insect welfare and industrial production, emphasizing the critical role of producers in shaping the future of insect welfare research within this values-driven industry.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Ingestion and egestion dynamics of micro- and nanoplastics in black soldier fly larvae

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Despite its importance for biowaste management and feed and fertilizer production, fundamental insights in the digestion processes of black soldier fly larvae (BSFL) are lacking. To gather additional data on the transition of different size particles, this study assessed the ingestion and egestion rates of various sized microplastics in the BSFL digestive tract. BSFL were exposed to a plant-based rearing diet (75% moisture content, 14% protein, 3% fat, 60% carbohydrates) spiked with a soluble dye (0.05% Blue1), two sizes of fluorescent microplastics (15 and 58 µm, ≈50,000 particles/g diet) and one size of palladium-doped nanoplastics (200 nm). During ingestion on the spiked diet (10-300 min) and egestion on the clean diet (60-1,080 min), larvae were regularly removed from the diet, dissected and the foremost dye and microplastics position (i.e. anterior, mid, posterior and hindgut) were determined. The dye position was determined with a stereomicroscope and the foremost microplastic position and total microplastic number with a fluorescent microscope. Nanoplastics were quantified in whole gut samples via ICP-MS detection of palladium-doped particles. All particles were ingested by BSFL, but discernible variations were observed based on particle size. The soluble dye passed through the digestive tract the fastest, followed by the 15 µm and the 58 µm microplastics, respectively. Ingested microplastic numbers were a magnitude higher for 15 µm (≈100/larvae) than 58 µm (≈3/larvae) microplastics. Relevant for product safety as animal feed, after 1080 min of egestion, larvae contained low amounts of 15 µm microplastics (4/larvae), while no 58 µm microplastics were present and nanoplastics were below the detection limit. Thereby this work contributes to fostering a deeper understanding of BSFL digestion with potential implications for improving bioconversion.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Dietary fat impacts nitrogen balance in black soldier fly larvae rearing

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The use of black soldier fly larvae (BSFL, *Hermetia illucens*) as a sustainable animal feed has gained significant momentum in recent years. Effective nitrogen (N) management is essential for maximizing nitrogen retention by the larvae during BSFL production. In our two prior independent studies, we observed that (1) specific larval densities and ambient temperatures contributing to high nitrogen retention often correlate with elevated gaseous nitrogen emissions and (2) the addition of dietary fat in rearing substrates may influence these emissions. This study investigates whether the inclusion of milk-based fat in the BSFL diet could enhance larvae performance at the population densities and ambient temperatures conducive to high N retention while mitigating N emissions. Our findings indicate that dietary fat increased yield and bioconversion efficiency. At the optimal population density for N retention, added fat not only reduced N emissions but also enhanced N retention in larvae. Similarly, added fat improved N retention at our optimal temperature setting but did not reduce N emissions. Overall, this study provides new insights into the impact of adding fat in the BSFL diet on the retention and emission of N during the BSFL rearing.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Black soldier fly larvae (*Hermetia illucens*) productivity can be optimized by amino acid supplementation

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An experiment was conducted to evaluate responsiveness of black soldier fly larvae (*Hermetia illucens*; BSFL) to substrates based on depectinized apple pomace (DAP). Analytical data of substrate, BSFL, and frass were used for mass balancing exercises. The trial comprised 4 nutritional treatments with 5 replicates each. Respective substrates contained either only DAP (T1), 70% DAP plus corn starch (T2), 84% DAP plus an amino acid mixture (AAM; T3), or 50% DAP plus corn starch plus an AAM (T4). With additions of starch and AAM similar starch and amino acid contents like commercial laying hen feed were achieved. The AAM included all 20 amino acids and overall amino acid profile of T2, T3, and T4 finally mirrored laying hen feed. BSF eggs were placed on substrates at constant temperatures of 27 °C. BSFL were harvested when ≥50% of BSFL per replicate achieved prepupae stage. Addition of both starch and AAM reduced age to harvest (T1 > T2 > T3 = T4). While addition of starch did not improve final biomass weight (T1 = T2), the combination of AAM with starch more than doubled the final biomass ($P < 0.05$). These results were mirrored in substrate conversion ($P < 0.05$). Fat yield was high in T2 and maximized in T4 ($P < 0.05$) suggesting that starch is mainly used for fat accumulation. While no free AA were detected in frass of T3 and T4, protein yield was maximized in T3 and T4 ($P < 0.05$) indicating that BSFL can well utilize free AA. However, although more than 2-fold higher in T4 compared to T1, T2, and T3 ($P < 0.05$) nitrogen (N) conversion (=N-BSFL/N-substrate) was still low (31%). Conversion of AA followed similar a pattern, but absolute levels differed between AA with lowest and highest values for Cys (15%) and for Lys (44%) in T4. Respective ranking of single AA conversions may allow for speculation on degree of performance limitation and offer opportunities to optimize dietary AA profile in order to maximise N-conversion and minimize N-excretions. Moreover, adjusting proportion of a well-balanced protein with starch then allows for optimizing key performance indicators such as time to harvest, biomass gain as well as protein and fat yield.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Optimizing black soldier fly growth through feeding rates and waste-based diets

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To enhance the profitability of black soldier fly rearing, it is necessary to improve both diet and diet/biomass ratio. For this purpose, 2 waste-based diets composed of Italian and Czech ingredients, along with Gainesville diet (ITA, REP and GA, respectively), were evaluated with four feeding rates: 0.4, 0.6, 0.8 and 1 g/larva. 6-days-old larvae were estimated and inoculated in varying number corresponding to the feeding rate into 10 kg of diet. To assess growth performance, larvae were sampled every 2 days, and harvesting occurred when the larvae growth between two sampling times was below 10%. At the end of the experiment, larvae weight was recorded, and bioconversion parameters were calculated, including bioconversion efficiency corrected for residue (BER), waste reduction index (WRI) and reduction rate (RR). Data were analysed by GLM model. Throughout the trial, weight was influenced by diets, feeding rate and time. The GA treatment displayed heaviest larvae, followed by ITA and REP ($P < 0.001$). Improved larval weights were also obtained with increasing feeding rate ($P < 0.001$). RR, WRI and BER differed among all groups, with GA showing the highest and REP the lowest values ($P < 0.001$). A greater RR was achieved by feeding the larvae with 0.6 g than 0.4 g per larva ($P < 0.001$), while no differences were observed for the others ($P > 0.05$). Larvae fed on 0.4 g each showed higher WRI when compared to the other feeding rate tested ($P < 0.05$), which were equivalent to each other ($P > 0.05$). The BER decreased with increasing feeding rates ($P < 0.001$). Based on the above reported results, it is evident that increasing the feeding rate and, as consequence, reducing the larval density, the bioconversion capacity of the larvae is deceased, even though larval weight appears to increase. Considering the interaction between diet and feeding rate, the optimal amount of diet is related to its nutrient availability. In conclusion, further studies will be required to correlate the nutrient composition of the diet with the feeding rate to provide comprehensive insights.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Utilization of spent coffee grounds as a feed additive for enhancing the nutritional value of *Tenebrio molitor* larvae

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The growing demand for sustainable protein sources has sparked interest in exploring alternative options that have a reduced environmental impact. This research delves into the utilization of spent coffee grounds (SCG), a widely available by-product, as a feed additive for Tenebrio molitor larvae. The larvae were fed with bran (their traditional feed) and a mixture of bran and SCGs (10 and 25% w/w). Various aspects such as larval viability, growth, and nutritional composition – covering protein, fat, fatty acids, carbohydrates, ash, carotenoids, vitamins A and C, antioxidant capacity, and polyphenols – were assessed. The study revealed that increasing the proportion of SCGs in the larvae's feed significantly enhanced their nutritional value. Notably, crude protein increased by 45.26%, vitamin C exhibited an 81.28% boost, and vitamin A experienced a remarkable surge of 822.79%. Additionally, polyphenol content increased by 29.01%. Furthermore, the oil extracted from these larvae displayed improved nutritional value and increased resistance to oxidation. These findings underscore the promising potential of using SCGs as a feed additive for *Tenebrio molitor* larvae, presenting a sustainable approach to enhance their nutritional profile. This research provides additional evidence supporting the idea that insects can efficiently ingest a diverse range of substances and effectively assimilate the nutrients present in their diet. Additionally, it is crucial to emphasize that *Tenebrio molitor* larvae have the capacity to emerge as one of the most nutritionally dense sources of animal-derived food. These observations underscore the need for heightened attention to their consumption, whether in their natural state or as supplements in low-nutritional value items.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Why my calculation failed: the impact of thermal acclimation and starvation on black soldier fly development

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The concept of accumulated degree days (ADD) and thermal performance curve are widely used in agricultural and forensic activities for estimating the duration of certain biological events. In this study, impacts of early-stage environmental temperature and starvation on black soldier fly *Hermetia illucens* L.) later stage development were determined. One hundred 7-d-old black soldier fly larvae were reared in a cup as a cohort, offered either 24 g (i.e. starved) or 34 g (i.e. fed) dry Gainesville diet, and allocated to either 20 °C or 30 °C. Larvae in 2/3 of the cups were transferred to 25 °C individually when the prepupal or pupal stage was reached, larvae in the rest 1/3 of the cups were kept continuously at 20 °C or 30 °C. Each individual was then monitored through adulthood. The date of transfer from one stage to the next was recorded for each, from which the developmental curve was determined. The developmental threshold and necessary ADD were calculated using the median duration in each cohort of each stage. At the end, the adult cold tolerance was determined by measuring the chill coma recovery time. As anticipated, lower early-stage environmental temperatures led to lower developmental threshold and greater ADD in later stages, which means, ADD is not a constant value and one cannot apply a fix ADD to populations with varied environmental backgrounds. Different developmental temperatures altered adult thermal tolerance, which indirectly revealed the biological impact of thermal acclimation on black soldier fly threshold temperatures. In addition, lower temperature and starvation led to greater variation in developmental duration, leading to synchronous development. Results in this study reveals the potential variation of developmental threshold and ADD. Attention should be paid to the historical temperature and feeding status when using ADD to estimate developmental duration.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Supplementing a high protein diet for black soldier fly larvae with minerals can improve their growth

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Black soldier fly *Hermetia illucens* (BSF) farming is not more than a decade old and the larvae nutritional requirements still remain unclear. Until now, most BSF nutrition trials in literature have tested one or several ingredients to feed larvae, but few have tried to delineate BSF actual requirements in nutrients, focusing on the ones supplying energy, namely protein, non-fibre carbohydrate and lipid. Conversely, BSF larvae needs in micronutrients, such as minerals, have never been studied to our knowledge although they play a critical role in animal physiology. Thus, we investigated whether supplementing BSF diet with polyhalite ($K_2MgCa_2(SO_4)_4 \cdot 2H_2O$; abbreviated PH), a pure mineral source, could improve BSF larvae growth performance. The BSF larvae were hatched at Entofood facility and fed a single nursery diet until five days post-hatch, then transferred to the experimental diets and harvested on the tenth day post-hatch. The control diet used was made of brewery by-products and rich in protein (35% of dry matter). On this basis, four others were designed to include 0.5, 1.0, 1.5 and 2.0% of PH. Larvae survival, growth, feed conversion ratio (FCR) and yield were estimated. The larvae and diets had their proximate and amino acids compositions determined along with their nitrogen content to assess the nitrogen emission for each diet. Larvae survival was around 100% for all diets and significant differences $P < 0.001$) appeared among them for growth, FCR and yield. For any of the diets containing PH, the larvae grew significantly more than for the control diet, and the maximal increase in growth (by 15.9% vs the control diet) was observed for 0.5% of PH. A decrease in nitrogen emissions proportional to PH inclusion was also estimated, from –5.3% (0.5% of PH) to –23.7% (1.5% of PH). No difference appeared in the larvae composition but for the ash content that was positively correlated with PH inclusion. Mineral supplementation in larvae diet is promising but needs further investigation. In particular, its influence may depend on the diet (here the diets were particularly rich in protein but poor in non-fibre carbohydrate) and on the larval stage.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Essential amino acid composition and supplementation in Gainesville diets impact larval development of *Hermetia illucens*

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Hermetia illucens (L.) (Diptera: Stratiomyidae), the black soldier fly, is a major species of interest within the insects as feed industry due to its ability to convert organic side streams into protein which can then be used as a feed within aquaculture, poultry, livestock, and petfood industries. Waste streams being used as feedstock for these larvae can have extreme variability when it comes to the nutritional composition, especially down to the amino acid level. This study was designed to determine the impact of supplemental essential amino acids on life-history traits of the black soldier fly using the standard Gainesville diet. Within this study, each of the ten essential amino acids were supplemented at a doubled rate and a tripled rate of what is naturally found within the standard Gainesville diet. Diets were placed in 50-mL Falcon tubes with 20 neonate larvae, each having 4 replicate tubes, and were run for the first 50% of the larval feeding stage based on accumulated degree hour calculations. Larval survivorship, weight and FCR were analyzed, and larval length and width were calculated using ImageJ software. Results showed that essential amino acid concentrations did impact life-history traits of *Hermetia illucens*. Micronutrients could serve as bottlenecks, or synergists, on black soldier fly growth. Data generated from this work could allow for such interactions to be identified and thus applied in the future to avoid pitfalls with varying organic side streams. Anticipated results will likely show that the supplementation of some of these EAAs will directly impact weight and FCR of the larvae, as indicated by other studies looking at supplementing tryptophan in honeybee workers. Survivorship is not likely to be impacted by these EAAs. Knowing how these micronutrients impact the weight of these larvae can have direct impacts on the mass rearing diet formulations in the future to help optimize the process.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Optimizing the rearing efficiency of the lesser mealworm, *Alphitobius diaperinus* (Panzer)

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The lesser mealworm has been authorized in the European Union as a source of food and feed protein. Yet, there are still several variables that could be optimized to increase the profitability of its mass rearing. A series of laboratory bioassays was performed to examine certain parameters that significantly affect the development of this species. We evaluated the suitability of sets of diets of 17.3, 22.5 and 27.5% protein, composed of different agricultural by-products of the cereal and legume seed cleaning process [i.e. triticale, barley, durum wheat, oats, peas, and lupin], as feedstocks for *A. Diaperinus* larvae. In another bioassay, agar agar, carrageenans, sodium alginate, guar or xanthan gum, pectin and carrots were evaluated as moisture sources. The optimum larval density was determined by comparing larval densities ranging from 7.5 to 53 larvae per cm². Finally, we compared the larval growth and feed conversion rate of seven geographically diverse *A. Diaperinus* populations (collected from poultry farms, provided by industrial producers, lab populations) when fed with flour from wheat bran, rice hulls or sunflower seeds. The agricultural by-products tested were shown to have the potential to serve as novel ingredients in insect diets to conventional feedstocks, thereby contributing to the reduction of the feed cost. The wet feeds tested may offer a more efficient means of supplying moisture to *A. diaperinus* compared to commonly used wet feeds. An optimal biomass efficiency can be achieved by using a larval density of 30.3 larvae per cm². The larval growth rate of the different populations in the various substrates varied, highlighting the need for genetic selection to achieve the best production output. The research work was partially supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 4th Call for HFRI PhD Fellowships (Fellowship: 11297) and by the COST Action "Improved Knowledge Transfer for Sustainable Insect Breeding (Insect-IMP), CA22140", supported by COST.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Effects of dietary iron concentration on growth, health and mineral distribution in edible insect larvae

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Edible insects are rising as a viable and sustainable alternative protein source, addressing the world's food security challenges. Alongside abundancy in essential macronutrients, edible insects are high in crucial micronutrients such as iron, which is vital for human health and particularly relevant given the common issue of iron-deficiency anemia. However, the influence of dietary iron concentration on edible insect larvae is an understudied field in entomophagy research. This study aimed to investigate the impact of dietary iron concentration on the growth, health and the macro and micro nutrients content in two prominent edible insect species, *Hermetia illucens* and *Tenebrio molitor*. Both species were reared on diets with added iron concentrations of up to 350 mg/kg using ferric ammonium citrate, and preliminary results indicated similar growth within this range. No dose-depended mineral alterations were detected in *Tenebrio molitor*. For *H. illucens*, further treatments were conducted at elevated concentrations reaching up to 3,500 mg/kg. Larvae fed on diets containing in excess of 350 mg/kg exhibited an increase in iron and calcium levels in a dose-responsive manner, alongside a reduction in zinc content. Additionally, larval growth was adversely affected at dosages above 900 mg/kg. Notably, at a dietary iron concentration of 3,500 mg/kg, *H. illucens* larvae developed distinct black lesions, akin to those observed in mammalian heavy metal toxicity, indicating a pathological response to iron overload. Despite variations in dietary iron concentration, protein distribution and content in both insect species remained consistent. This research demonstrated a dose-dependent relationship between the growth and nutritional facts of edible insects and the levels of dietary iron present in their feed, offering data that could enhance the nutritional profile of insect-based foods.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

BSF larvae reared on EU-authorized and unauthorized biowaste can bioaccumulate γ-tocopherol and carotenoids

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Food loss, food waste and agricultural by-products have become key targets in the circular bioeconomy objectives in the EU. The exploratory project FLY4WASTE aims at assessing the benefits and risks of the insect-based bioconversion of these biowaste by the black soldier fly larvae (BSFL), *Hermetia illucens*. Indeed, it can convert a wide variety of biowaste and be used as a new feed source. In this experiment, BSFL were reared on EU-authorized agricultural by-products; wheat bran, carrot, apricot, salad and a wheat bran-apple mix used in the industry (standard) and unauthorized biowaste such as school canteen and supermarket waste. BSFL weights, total protein and lipid contents were measured. A total of 16 fat-soluble vitamins and dietary carotenoids were also screened: retinol, retinyl palmitate, cholecalciferol, ergocalciferol, ergosterol, α and γ-tocopherol, α and β-carotene, lutein, lycopene, zeaxanthin, β-cryptoxanthin, astaxanthin, phytoene and phytofluene. They were extracted using a double extraction with hexane and quantified by HPLC-UV. Fresh weights of BSFL significantly differed: BSFL reared on apricot and supermarket waste had lower weights (85 ± 4 and 90 ± 4 mg, respectively) than their counterparts (120 ± 7 mg for standard). However, the dry weight, protein and lipid contents of the larvae were similar in the different BSFL groups. Nine bioactive molecules were detected in the substrates and BSFL. For some molecules, bioaccumulation in BSFL was observed; up to 55 times the concentration in the substrate (β-cryptoxanthin in school canteen waste). Conversely, vitamin A, D and astaxanthin were neither detected in the substrates nor in the BSFL. Their absence could be explained by their too low levels in biowaste or their destruction during biowaste treatment. Thus, it is possible to enrich BSFL in fat soluble micronutrients by rearing them on EU-authorized and unauthorized biowaste containing these compounds without compromising on their macronutrient qualities. It is also a promising work on the use on yet unauthorized biowaste in the EU for the insect industry.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Optimization of the adult performance and larval growth of the superworm, *Zophobas morio*

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Among the insect species that have been used for commercial production, the superworm, *Zophobas morio* (F.) (Coleoptera: Tenebrionidae), has considerable potential for exploitation for food and feed applications. Research though on this species and the optimization of its production processes has been rather limited. Therefore, further experimentation is needed to fully unfold the potential of *Z. morio* as a nutrient source. Along these lines, the aim of the present study was to optimize the adult performance and larval growth of *Z. morio*. In order to enhance the reproductive output of adults and define the optimal adult ratio for breeding, three different female:male ratios were studied, i.e. 50:50, 60:40 and 80:20, in a laboratory bioassay, by weekly evaluating the adult survival, the number of eggs laid per female and the egg hatching rate for a total period of 13 weeks. In a second series of bioassays, the effect of the diet protein content on the growth of *Z. morio* larvae was studied by rearing them on wheat bran–based substrates with increasing percentages of dry yeast and subsequently ascending protein contents, i.e. 15.2, 20, 22.5, 25, 27.5 and 30% protein. Finally, in a third bioassay we evaluated the growth of larvae fed isoproteinic wheat bran-based diets (20% protein) with different protein sources, i.e. dry yeast or pea, egg white, brown rice and soybean protein powders. Based on the results, the female:male ratio can significantly affect the reproductive output of *Z. morio* adults, the highest cumulative number of eggs per female being recorded for the 50:50 ratio. Similarly, the diet protein content, affected larval growth, the optimal larval development being achieved when larvae were fed a diet with 20% protein. Finally, among the isoproteinic diets tested, the worst performance, in terms of larval growth, was recorded for larvae fed on the wheat bran diet supplemented with the pea protein powder. The results of the present study aim to contribute to the optimization of the rearing process of *Z. morio* and the better understanding of its nutritional requirements which still are largely unknown.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Filling a gap in diet formulation approach: how to assess digestibility in black soldier fly larvae (*Hermetia illucens*)?

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Black soldier fly larvae (BSFL) growth, protein and lipid contents vary with the profile of their feeding substrate. To mitigate substrate seasonal variability and standardise production, large-scale rearing units mix diverse ingredients to formulate diets with similar nutritional profiles. However, variability in larval performance persists, likely due to differences in digestibility. Two approaches are proposed to assess digestibility coefficients in BSFL, and tested on chicken feed (CF), corn gluten feed (CGF), discarded potatoes (DP), wheat bran (WB) and wheat distillers grain (WDG). The asymptotic estimated digestibility (AED) approach relies on the use of increasing larval density to reach total substrate ingestion. Comparing the nutritional contents of distributed feed and frass allows to calculate the maximal nutrient fraction that BSFL and their microbiota can digest. Our results identified the values of 80.4 ± 0.9%, 45.6 ± 0.5% and 71.6 ± 0.7% for AED of dry matter, 99.3 ± 0.7%, 100.0 ± 0.2% and 84.3 ± 0.6% for AED of starch, and 79.6 ± 1.2%, 78.6 ± 0.6% and 44.2 ± 1.5% for AED of protein, in CF, CGF and DP, respectively. The same method can be used with fixed larval density and increasing feeding time. The approximate digestibility (AD) approach involves incorporating an indigestible marker in the substrate and collecting BSFL excreta in a clean container. AD is calculated based on marker concentration in feed and excreta. AD of dry matter was 87.5 ± 1.2%, 64.2 ± 2.6%, 77.1 ± 1.1%, 57.9 ± 2.1% and 86.1 ± 1.2% in CF, CGF, DP, WB and WDG, respectively. Pros and cons of each approach are discussed. Further aspects need to be explored, such as additivity of AED or AD, before digestibility can be implemented in BSFL diet formulation strategies.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

The first results of analysis of residues in mealworms and Jamaican field crickets fed by carrot treated by pesticides

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As the published data on hazardous chemicals in reared insects as well as data on accumulation of chemical contaminants from the substrates are very limited, we decided to investigate the occurrence of pesticide residues in the laboratory-farmed Jamaican field crickets and mealworms. The experimental insects were provided by carrots, which were treated with pesticides (containing fluazifop, pirimicarb, azoxystrobin, difenoconazole, and tebuconazole) once or twice during their growth. The control groups were fed by untreated carrots. Then, the presence of pesticide residues in insect samples was analyzed using high-resolution mass spectrometry. It was demonstrated that both Jamaican field crickets and mealworms took up fluazifop and tebuconazole. Long-term exposure to carrots with incurred residues of these pesticides led to the formation of conjugated or bound residues in insects. Fluazifop-P concentrations were in the range of 0.004-0.014 $mg \cdot kg^{-1}$ in crickets and in the range of 0.003-0.006 $mg \cdot kg^{-1}$ in mealworms. Residues of tebuconazole were detected only in the samples of crickets (0.001-0.070 $mg \cdot kg^{-1}$). A statistically significant difference was found between the insects fed by the carrots treated with pesticides once and twice in the case of fluazifop (both insect species) and tebuconazole (crickets). After starvation of insects, commonly used before processing edible insects, a significant decrease in residue concentration was observed. Nevertheless, no sample of tested insects was considered non-compliant after taking into account the measurement uncertainty even though the residue concentration of fluazifop-P in six samples of crickets exceeded the MRL (0.01 $mg \cdot kg^{-1}$) from Regulation (EC) No.396/2005. Supported by METROFOOD-CZ, MEYS, LM2023064, GAČR, INPROFF, 21-47159L, and by the Ministry of Agriculture NAZV, QK23020101.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Fermentation pre-treatment of lignocellulosic biomass for black soldier fly feed production

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Abundant lignocellulosic sidestreams represent a possible low-cost substrate for BSFL, but feed conversion ratios (FCR) reported in the literature are low. This is due to the inability of BSFL to digest cellulose, as increasing inclusion of cellulose in BSF feed trials with soybean okara shows linear FCR increases (Figure 1). In contrast, supplementation of Single Cell Protein (SCP), which can be derived from cellulose through microbial bioconversion, decreases FCR, on account of the higher protein content and digestibility (Figure 1). Here, food grade organisms *Cyberlindnera jadinii* and *Yarrowia lipolytica* were cultivated to produce a microbial biomass slurry which did not require drying for use as BSFL feed. With a crude protein content of 40 to 42%, these slurries were shown to be an effective replacement for soybean okara (25% crude protein). This approach has now been applied to lignocellulosic biomass from food manufacturing to upgrade protein content using a two-stage fermentation approach: thermophilic anaerobic fermentation with a cellulolytic consortium followed by aerobic mesophilic acetate fed-batch fermentation with *C. jadinii*. 25% of fibre was converted into SCP with a concurrent crude protein increase from 9.3% to 28.3% on dry matter basis. BSFL feed trials (300 g substrate; 150 5-DOL; 9 day rearing) with the fermented residues achieved a FCR of 6.6 ± 0.7 significantly lower than untreated biomass with FCR 8.9 ± 0.7, but not as low as the reference feedstock with FCR 4.5 ± 0.4 (Figure 2). Fermentation pre-treatment can significantly improve BSFL rearing outcomes on lignocellulosic biomass, and with further improvement in fibre conversion efficiency could enable the use of low-cost substrates for commercial BSFL rearing to address the high cost of production.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Physical attributes first: a new approach of feed formulation to improve BSF larvae (*Hermetia illucens*) performance

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The utilisation of multiple agri-wastes within a single diet for black soldier fly larvae (BSFL) is a common strategy to provide essential nutrients for the larvae. Formulators aiming to produce a uniform diet from various wastes must consider the individual feedstock's nutritional attributes and physical attributes, such as viscosity and water retention capacity. However, conventional feed formulation prioritises nutritional attributes above physical ones. This approach is based on legacy knowledge systems from large animal nutrition and may fail to account for unique attributes of BSFL biology and feeding behaviour. In BSFL the feed is a source of nutrition for larvae and substrate microbiota but it also serves as the insect's physical environment, a key difference from conventional animal livestock. This study explores a novel approach to feed formulation by prioritising the physical attributes of BSFL feed as a formulation strategy. The research focuses on understanding the diverse dimensions of feed formulation, including particle size, feed texture, and rheology to identify factors influencing the growth and development of BSFL. We first classified feedstocks into dry and moist materials, along with subgroups based on their textures and rheological properties. The final diets were then formulated and classified based on the percentage inclusion of the main groups and the structure type of the moist materials. Our results indicated a 45% total inclusion percentage of dry materials coupled with a fibrous-structure moist material in the formulation played a crucial role in achieving both 4 days fewer rearing days and significantly increased larvae yield by 11.38% ($P = 0.011$) – a dual impact. Additionally, controlling the feed structure could impact the bioconversion efficiency rate (23.3%, $P = 0.0048$) and ease harvesting on Day 10 of growth out. Overall, this focus on formulation prioritising physical attributes of feedstocks has the potential to optimise outcomes by properly taking BSFL biology, microbial ecology, and feeding behaviour into account in formulation.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

A digital twin solution for insect-based bioconversion

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Industrialized insect-based bioconversion is deemed to be an economically and environmentally promising approach for solving protein shortage and organic waste treatment issues worldwidely. Its overall idea is to first feed insect larvae on pretreated organic waste. Then, when the larvae are well developed, they will be harvested and processed into high-value insect products that can be further used as food and feed. As such, the massive energy and nutrients in various organic waste streams can be efficiently recycled back into human food chains. Now, the challenge is how to effectively realize it. This work proposes a turn-key solution to automate the industrialized rearing and mass production of black soldier fly (BSF). The overall solution is modularized into 5 subsystems: a climate-controlled container-based rearing system, an AMR transport system, a robotic material handling system, a product processing system, and a digital twin (DT) dashboard. During the engineering phase, each subsystem's mechanical structure is first designed. Then, their individual DT models are built and assembled to dynamically simulate and verify the resultant system's functionality and productivity. Once deployed, the DT dashboard enables real-time monitoring, controlling and management of the physical bioconversion process by aggregating and visualizing its operation status, rearing schedules, maintenance plans and all related environmental data. The proposed solution has been successfully deployed and is continuously operating in a commercial-scale pilot plant in Dongguan, China. The pilot plant consists of a breeding centre, a bioconversion centre, and a circular bioeconomic showroom. It can handle up to 55.44 tons of organic waste for each batch of BSF larvae. Thanks to DT technologies, the solution's modular, reusable and scalable design allows for easy reconfiguration, fast expansion and continuous optimization. In conclusions, the proposed industrialized BSF bioconversion system offers a sustainable solution for organic waste management and alternative protein production. Further research and standardization efforts are needed to fully realize its potential and integrate it into larger smart agriculture systems.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Substrate moisture content and relative humidity affect growth and gaseous emissions in black soldier flies

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Black soldier fly larvae require a high moisture content of the substrate, while at the end of the process this needs to decrease for automated harvesting. The drying process is influenced by scale, initial dry matter (DM) content and relative humidity (RH), with likely consequences for larval development and gaseous emissions. A large scale (300 kg of substrate in five trays per replicate) experiment was conducted to quantify these effects, varying starting DM contents (31 vs 34%), and RH (50 vs 70%). During 13 days samples of larvae and substrate were taken daily and analyzed for DM, N, and fat. Substrate pH and NO_2 emissions were determined daily, while substrate temperature, O_2 consumption, CO_2, CH_4, and NH_3 production were measured continuously in our climate respiration chambers. Initial growth was higher at the higher DM and then lower in the last three days. A higher RH increased growth in the middle of the growth period. Larval N content decreased over time and was higher in the high DM (D3-13). Fat content increased over time and more so in the high DM (D3-11). Substrate temperatures increased over time, with initially higher temperatures in the high DM (D1-9) and higher RH (D0-4). Substrate DM increased over time and stayed higher for the higher DM. This difference increased over time and a lower RH increased the drying process (D8-13). During the experiment pH increased, and this increase was quicker for the high DM (D6-11). A higher RH led to a slightly higher pH at D9-11. Final tray yields were higher for a lower DM (3.5 vs 3.8 kg) and less substrate was leftover (7.3 vs 9.2 kg). Greenhouse gas and NH_3 emission patterns were affected by both DM content and RH. The higher DM led to earlier and lower peaks of CO_2 emissions. Higher DM and higher RH increased NH_3 emissions, whereas the opposite was true for CH_4. In conclusion, this is the first large scale study reporting the effect of both RH and moisture content on production characteristics and gaseous emissions during a full rearing cycle. The collected data can be used to find an optimal balance between larval production, sievability, and gaseous emissions.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Could your weekend beer ... save the world? The use of brewing waste in cricket diets

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Access to sustainable and cost-effective feed is a major impediment to large-scale insect production. To offset environmental and economic costs, organic waste products and other sustainable feed options may act as suitable alternatives to traditional, less-sustainable feed ingredients. Before novel feed ingredients can be implemented, we must determine how they compare to existing farm feeds. We monitored the performance (e.g. growth, development time, weight, and survival) of a popular farmed cricket species (*Gryllodes sigillatus*) reared in isolation on diets containing a waste product from brewing beer (spent grain) (n = 27 crickets per diet). Our findings reveal that spent grain can be used as a replacement for fishmeal (a primary protein source in many existing farm feeds) with minimal impacts on final body mass ($P = 0.0304$) and development time ($P = 0.0324$), and no impact on survival or whole-body macromolecule content (nutritional quality). Given the seeming utility of using spent grain as a suitable alternative to traditional cricket feed, we are conducting a multigenerational experiment that compares cricket performance on a high-inclusion (75%) spent grain diet against a control (existing farm feed). We also included a treatment that increased the spent grain inclusion rate from 15% to 75% (increasing by 15% each generation) to evaluate how performance is impacted. Current results indicate that crickets reared on the 75% spent grain diet are about 21% smaller ($P < 0.001$) and 15% smaller ($P < 0.001$) at a harvest compared to the control for generations one and two, respectively. However, crickets reared on the gradual inclusion spent grain diet (15-30% spent grain) did not differ from the control crickets. Overall, these studies demonstrate that tropical house crickets are able to convert sustainable feed sources like spent grain with minimal impacts on product yield and quality, which extends the applicability of insect production to the circular economy.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Physical properties of insect diets: does dietary particle size influence cricket life history?

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Artificial diets are critically important to the success of mass rearing insects, as diet is an expensive production component. One way to improve feed efficiency is through dietary particle size optimization. We used a commercially reared species, *Gryllodes sigillatus*, to test whether individual crickets reared from hatch to adulthood on different particle sized diets would grow differently. Crickets fed ≥0.5 mm diet grew heavier during the first three weeks but weighed the same after six weeks regardless of diet size. We then provided crickets with a choice of particle size throughout development to test for dietary size preference. Given a choice, crickets consumed the most food from the 1.0-1.4 mm diet. Crickets also preferentially select ingredients from mixed diets, so to test whether grinding a conventional diet to a finer particle size could influence performance traits, we ran a larger scale group rearing experiment and found no effect of grinding on mass gain or development time. Pelleting diet is another method for eliminating self-selection of ingredients, and so we tested whether pelleting finely ground conventional cricket feed would result in any substantial changes to the developmental life history of individual crickets. Crickets fed a 2 mm pelleted diet grew larger body size but were not significantly heavier. Overall, our results demonstrate that particle size optimization can be leveraged to enhance cricket life history traits important to mass production, but there is still much to learn to reach a predictive understanding of how to optimize particle size in cricket diets.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Metabolism and growth of BSFL under factory and laboratory conditions

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Considering the need for optimization and monitoring of large-scale production of black soldier fly larvae (BSFL, *Hermetia illucens*) for food and feed, we conducted a series of respirometry experiments under both large-scale commercial conditions and small-scale laboratory conditions to investigate effects of rearing temperature and diet composition. In our initial experiment at ~35 °C, we validated that our small-scale respiratory setup (0.4 kg of feed, 600 BSFL) generated similar data on growth, survival, metabolic rate, feed conversion and body composition as in large-scale growth stables (>8,000 kg of feed, >12 million BSFL). Using our small-scale setup, we then investigated the impact of temperature (27 °C to 42 °C) during a 7-day rearing period. The continuous respiration measurements revealed faster development through the final larval instars at higher temperatures, but after the 7-day growth period, total larval biomass was high at temperatures ranging from 27 °C to 39 °C, while 42 °C severely reduced growth and respiration. We found rearing at 27 °C to result in marginally higher larval biomass, higher protein content and reduced CO_2 emissions per gram larvae. In a final set of experiments with the small-scale setup, we investigated variations in dietary protein to carbohydrate ratios during a 7-day rearing period. We found strong positive correlations among protein content, metabolic rate and total growth, and high protein diets also produced leaner larvae. The respiratory gas exchange ratio (RER, CO_2 production/O_2 consumption) correlated strongly with larval lipid content as this ratio is reflective of both catabolic (maintenance metabolism) and anabolic (growth and lipogenesis) processes. In conclusion, continuous measurements of gas exchange from BSFL production systems holds considerable promise to effectively monitor developmental rate, gross larval production and larval body composition.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Maximizing performances of *Hermetia illucens* larvae on industrial diets by optimizing protein and carbohydrate content

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Black soldier fly larvae (BSFL), *Hermetia illucens*, can efficiently convert organic substrates into insect biomass, which is suitable for livestock feed. The performance of BSFL depends on the macronutrient composition of their diet, and especially on protein (P) and carbohydrate (C) content. This study uses a geometric approach to determine the effect of different ratios between P and C concentrations in semi-industrial diets formulated with cereals and tubers by-products typical from North-East of France (grain, potato, corn, beetroot). Seventeen oligidic diets varying in their P and C concentrations were formulated, with C+P concentrations ranging from 25% to 65% and C:P ratios from 1:1 to 4:1. These diets were used to feed the BSFL for seven days. The impact on the feed conversion ratio (FCR), protein and lipid content of 14-day-old larvae (14 DOL) was assessed. Initial feed provided and frass were also analyzed to account for estimated digestibility of P and C. Our results indicate that BSFL performance is more affected by C+P concentrations than C:P ratios. The best-performing diets for minimizing FCR were those with a C+P concentration superior to 45%, while the 14 DOL protein plus fat content was maximized with diets containing approximately 50% C+P and a C:P ratio between 2:1 and 4:1. These findings complement the results already available in the literature for artificial diets by examining semi-industrial diets on a more restricted range of P and C, and considering the estimated digestibility of these macronutrients. They also confirm the importance of the ratio of dietary protein and carbohydrate to optimize BSFL performance on an industrial scale. Future studies should also investigate optimal ratios of the different types of carbohydrates and amino acids to further explore levers for optimizing performances of BSFL.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Effect of dietary protein in isoenergetic diets on energy and nitrogen digestibility and retention of black soldier fly

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Black soldier fly larvae (BSFL) efficiently convert organic substrates into insect biomass, suitable as sustainable livestock feed. To optimize BSFL production, it is important to determine the ideal protein content in the diet. This study evaluated the effects of 5 practical and isoenergetic diets (PID) with decreasing carbohydrate (C) and increasing protein content (CP) (44:10, CP10; 40:12, CP12; 30:16, CP16; 26:20, CP20; 20:24, CP24) on larval growth performance and conversion process of biomass. A total of 350,000 6-day-old BSFL were reared in a controlled climate chamber (28 °C, 55% RH) and randomly assigned to PID (n = 5/group), with the Gainsville diet (GD) as environmental control. PID (25% DM) were composed of side streams. PID and BSFL were analyzed for their dry mass (DM), gross energy (GE) and nitrogen (N); CP was calculated. Data were analyzed using R (Version 4.3.1) by ANOVA. Data were used to measure average daily gain (ADG), development time (DT), asymptotic estimated digestibility (AED), retention of DM (DMR), gross energy (GER), and CP (CPR) of the diets converting into BSFL biomass. The GE content was 17.5 ± 0.3 MJ/kg DM across all diets. ADG (R2 = 0.74; $P < 0.001$) were quadratically affected by dietary protein content, with CP16 larvae having the highest values and CP24 the lowest. There was a linear effect of DT of the CP12 group, which was increased by 35% compared to CP24 (R2 = 0.84; $P < 0.001$). AED – as an indicator for reduction in feed and corrected by residues – of N showed a quadratic effect with 30% higher values in group CP12 than CP24 (R2 = 0.75; $P < 0.001$), while AED of DM and GE were not affected by diets ($P > 0.05$). Diets quadratically affected the retention – as a conversion factor from feed to BSFL biomass – of DM in larvae (R2 = 0.55; $P \leq 0.001$), GE (R2 = 0.84; $P < 0.001$) and N (R2 = 0.92; $P < 0.001$) with the highest values observed in CP16 and the lowest in CP10 and CP24. In conclusion, an unbalanced dietary C:CP intake is associated with reduced biosynthesis that affects, among other things, larval performance, development, and conversion efficiency.

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Feed colonization rate by different sizes of BSF larvae

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The feeding performance of black soldier fly (BSF) larvae is dependent on their ability to colonize the feed substrate. Although there is abundant literature regarding the feeding performance of BSF larvae on different types of feed, there is a gap in understanding how larval size affects their feeding behaviors and the rate at which they can colonize the substrate. To address this, a study was conducted to evaluate the feeding speed of BSF larvae of standard age but of different sizes on feed placed inside a vivarium made from a transparent acrylic feeding container, known as Dipterium. Three different categories of larvae, Small, Medium, and Large, were used, and their behavior was observed daily until one of the categories reached the bottom of the Dipterium. The speed of the larvae feeding was calculated based on the depth that the larvae burrow into the feed each day. The results of the study show that, compared to Small-sized larvae, the feeding speed (cm/day) of Medium and Large larvae increased by 17% and 21%, respectively. Therefore, it can be concluded that the size of the larvae affects their substrate colonization behavior, as larger larvae can consume more feed and reach fresh feed at depth faster than smaller larvae given the same amount of time. The study also found that even though their feeding rate increases, the growth rate of the larvae slows down as they increase in size. These findings can have significant implications for the study of BSF larval biology, as they provide valuable insights into the feeding behavior of larvae and its impact on their growth rate.

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Sofia diet: a standard diet for black soldier fly research and development

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With the growing interest in *Hermetia illucens* as an insect model for bioconversion, a common ground for scientific evaluation is crucial. The traditional control BSF larva diet is based on raw ingredients subject to seasonal fluctuations, regarding supply. Furthermore, the properties of its components impact the chemical and physical composition of the diet. As this variability leads to unfair comparisons, there is an emergent need in the field for a stable BSF recipe that can be easily replicated worldwide. The objective of this study is to engineer a specialized diet for BSF, containing standard and quality-wise stable reagents, which are globally available. More than 40 different recipes were designed and evaluated through a mixture design experiment. A protocol for feed preparation was also developed. The test diets contained stable ingredients: hydrolyzed yeast, sucrose, sunflower oil, jellifying agent and water. Results indicate that larval growth and survival are significantly affected by the protein content and texture of the diet. BSF larvae, reared on our proposed "Sofia diet", reach at least 200 mg of individual weight on average and the feed handling was easier compared to other diets. This proposed, easy to replicate "Sofia diet" will be a powerful tool in both academic and industrial environments. Moreover, its application as standardized control will allow researchers around the world to compare strains and rearing conditions, assess effects of new ingredients and provide a solid foundation for fruitful scientific collaborations.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Potential multigenerational effect of different diet profiles on black soldier fly fitness

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Multigenerational effects can be observed in response to changes in diet, with those including changes in body size, development rate, survival, and fitness of offspring. The goal of this study was to assess whether different diet profiles provided during larval stage would impact fecundity and fertility of black soldier fly adults across three generations (G1, G2 and G3). Three different diet profiles (treatments) were offered with crude protein levels ranging from 12-21% . Larvae were reared at the same density, feed ratio (0.25 g/larvae), and environment conditions (27 °C /55% RH) across the three treatments. To assess fertility and fecundity, a minimum of twenty 24h-old virgin males and females were sampled per treatment/generation and kept isolated in mason jars for 48 h. Sperm was collected through mechanical induced ejaculation and diluted in Kiev buffer solution. Overall sperm count and viability data were collected using the Nexcelom Cell Counter. Sperm motility was assessed using the OpenCASA software connected to a ZEISS Axioscope 5 microscope. Ovaries were removed through dissection and the number of developed eggs were counted. Moreover, total daily egg mass, hatchability, and egg individual weight were recorded for the entire reproductive cycle. Hatchability was 96.37% ($P = 0.0006$), with an average of 980 eggs per female ($P = 0.0020$), for the treatment with the highest protein content. The results found may allow insect farmers to estimate the impact of brood diets on the colony fitness across generations, and consequently, implement strategies to enhance productivity and reduce cost without compromising product safety and quality.

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Application of the Gompertz growth model to black soldier fly experiments

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Evaluating the influence of different dietary treatments on animal growth typically entails the construction of growth curves. Larval growth, like many biological phenomena, adheres to a sigmoidal pattern. While nonlinear models, such as the Gompertz model, have been widely applied in the analysis of growth curves, past iterations have encountered challenges related to applicability and parameter interpretability. Addressing these limitations, the U-Gompertz model, an extension of the Richards family proposed by Tjørve and Tjørve (2017), offers alternative formulations with easily interpretable parameters, including valuable insights related to growth rate dynamics. This is particularly important in the context of time-treatment interactions, where linear models often prove inadequate. In a study involving the evaluation of different feedstock manipulations of an organic waste feedstock for feeding black soldier fly larvae, a series of experiments using 300 3-day-old larvae, samples of 10 larvae with replacement every two days, to build the growth curve, we applied the U-Gompertz model to assess the impact of different feedstock treatments on black soldier fly larval growth. This approach yielded three crucial absolute parameters: maximum weight, maximum growth rate, and time to growth curve inflection. The U-Gompertz model, employed for statistical analysis with graphical representation aided by confidence intervals, emerged as an interesting choice for such experiments. Notably, it consistently mirrored observed patterns and trends, showcasing resilience even under conditions of limited replication. For this type of analysis, we recommend increasing measurement frequency to enhance result accuracy. Overall, the U-Gompertz model stands out as a potential valuable tool for researchers seeking precise and reliable insights into the growth dynamics of black soldier fly larvae in diverse dietary environments.

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Optimal dietary protein levels for black soldier fly larvae (*Hermetia illucens*)

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The impact of different crude protein (CP) and amino acid (AA) levels in substrates on black soldier fly larvae (*Hermetia illucens*; BSFL) performance was studied. As examining AA requirements of and utilization by BSFL is not possible using classical feeding studies, modelling AA flows by mass balancing offers an alternative approach. The trial comprised 5 treatments (5 replicates each). Substrates were based on depectinized apple pomace (DAP), corn starch plus an AA mixture, and contained 19.3 (T1), 16.9 (T2), 14.5 (T3), 12.2 (T4), and 9.8% CP (T5). Lysine was varied with CP but overall AA profiles (Lys = 100%) were similar in all treatments. 100 BSF neonates were placed per replicate, while samples of 25 larvae were weighted in 4-day-intervals. BSFL were harvested when ≥50% of BSFL per replicate achieved prepupae stage (days 24/25). Average individual BSFL weight peaked at day 16 except for T5 which peaked at day 12. Final biomass was maximized in T4 and was lowest in T5 ($P < 0.05$), while the other treatments were in between. Substrate conversion was lowest in T4 and highest in T5 ($P < 0.05$). Fat yield decreased linearly from T1 to T4 by 13.9 mg per % CP ($r^2 = 0.99$) whereas fat yield in T5 was 71% lower than in T4 ($P < 0.05$). Protein yield was 37% lower in T5 compared to T4 ($P < 0.05$), while protein yields of T1 to T4 were similar. Nitrogen (N; 41%) and AA conversion (Ø32%; 18-46%) into BSFL biomass (=BSFL/substrate) were highest in T4 ($P < 0.05$). N- and AA-losses (=(substrate-BSFL-frass)/substrate) linearly decreased from T1(N: 31%; ØAA: 61%) to T5 (N: 5% $r^2 = 0.95$; ØAA: 33% $r^2 = 0.97$). Utilization (=BSFL/(BSFL + frass)) of N linearly increased from T1 (46%) to T 5 (86%, $r^2 = 0.94$) but maximized for AA in T4 (Ø46%; 22-84%; $P < 0.05$). No free amino acids were detected in frass suggesting 100% digestibility of free AA while AA levels in frass were similar to DAP-AA. It is concluded that BSFL require at least 12.2 % CP for optimal productivity, AA-conversion and AA-utilization of AA. While N-losses (ammonia) can be minimized, differences between AA in terms of conversion, losses and utilization also offer opportunities for dietary AA optimization.

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Consideration of supplements to promote the growth of black soldier fly larvae

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The black soldier fly larvae (BSFL; *Helmetia illucens*) feeds on a wide variety of food wastes, including kitchen scraps, and on livestock manure. BSFL have excellent nutritional value with potential for use as a component of livestock and fishery feeds. Large quantities of spent coffee grounds as waste from industrial beverage manufacturers are difficult to recycle. BSFL reared solely on coffee grounds exhibited slow development, with a 75% decrease in weight during the final instar compared to those fed with the standard feed. In this study, we examined the use of spent coffee grounds as supplementary food to improve the rearing environment of BSFL when mixed with bean curd, breadcrumbs, and vegetable scraps, which are unsuitable alone for rearing BSFL. The survival rate of final instar larvae reared on a mixture of bean curd and coffee grounds (3:1 by weight) increased from 30% on bean curd alone to 95%, with an average weight increase of 183%. BSFL reared on breadcrumbs alone exhibited slow development; however, when mixed with coffee grounds (3:1 by weight), they developed normally, with final instar larvae 65% larger than those fed on breadcrumbs alone. In these diets, the coffee grounds created air gaps, which helped to soften the diet's viscosity, enabling the larvae to move freely in the diet and facilitating development. Although larval development did not improve when coffee grounds were mixed into vegetable scraps, the number of final instar larvae increased, suggesting a positive effect of coffee grounds on larval survival. While the growth of BSFL reared on coffee grounds alone is poor, growth on coffee grounds mixed with other food wastes is good. Our findings suggest that used supplementary feed can enhance the dietary environment and promote larval growth. Therefore, spent coffee grounds as supplementary food could become promising nutritional components in the production of BSF.

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Impact of supplemental tryptophan and zinc on the performance of *Hermetia illucens* and *Cochliomyia macellaria* larvae

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Within the mass reared insects as food & feed industry, macronutrient (e.g. protein, carbohydrates) impacts have been heavily studied; however, there is a major knowledge gap when it comes to micronutrients (e.g. essential amino acids). Feedstock optimization for insects is vital to this industry to create high protein insects, and a study on the supplementation of tryptophan in *Apis mellifera* (Hymenoptera: Apidae) diets has been shown to increase protein content, survivorship, and weight of worker honeybees. Tryptophan and zinc have also been shown to be physiologically linked, showing the uptake of one micronutrient can directly impact another. This study determined the impact of a doubled concentration of tryptophan, zinc and a combination of both on the standard diets of *Hermetia illucens* (Diptera: Stratiomyidae) and *Cochliomyia macellaria* (Diptera: Calliphoridae), based on the amount naturally found in those standard diets. Diets were made in 50 mL Falcon tubes, with 20 larvae in each tube, each having 10 replicates. All experiments were conducted at 27 °C, 70% relative humidity, and were run for the first 50% of their respective larval feeding stages, based on accumulated degree hour calculations. Larval survivorship, weight and feed conversion rate were analyzed, and larval length and width were calculated using ImageJ software. Results have shown that BSF are more impacted by these nutrient changes, showing an almost 25% weight increase with the supplementation of tryptophan, which can be explained by the difference in life-history strategies between the two species. Understanding the impacts of micronutrients on insect growth could allow for diets to be formulated a finer scale rather than simply on protein or carbohydrate content.

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Micronutrients impact life-history traits of *Cochliomyia macellaria*: relevance to mass produced insects

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Concentrations of tryptophan vary within tissues of a single carrion or decaying plant material, and the impact of these concentrations on the life-history traits of *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) has yet to be studied. The uptake of one micronutrient can directly impact another, as it was shown that tryptophan and zinc are physiologically linked. The impacts of these micronutrients on life-history traits of dipteran larvae can have applications in not only forensics, but in insects as food & feed industries, where diet optimization is a necessity. *C. macellaria* has been mass reared as a protein source, and also as a model for SIT programs for its sister species, *C. hominivorax*. This study was conducted using an artificial Holidic diet (Piper *et al.*, 2014) where 15 diets with differing concentrations of tryptophan increasing at 1.25%, 15 diets with differing concentrations of zinc increasing at 1.5%, and combination diets with simultaneous differing amounts of tryptophan and zinc were examined for their impact on *C. macellaria* development and survivorship. All experiments were conducted at 27 °C, 70% relative humidity, and were run for the first 50% of the larval feeding stage, based on accumulated degree hour calculations. Larval survivorship, weight and FCR were analyzed, and larval length and width were calculated using ImageJ software. Results indicate that survivorship was not impacted, however weight was increased up to 50% when tryptophan was increased. Zinc did not seem to impact weight gain of larvae, or have a negative impact on survivorship. Tryptophan levels did have a greater impact on weight and FCR of larvae, showing the importance of studying these micronutrients further.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Iso-nutrient and waste-based diets for yellow mealworm

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Tenebrio molitor larvae (TML) are usually grown on cereal products, which already find application in feed industry. In the frame of the PRIMA2021 ADVAGROMED project, the influence of iso-nutrient (gross energy, crude protein (CP), and ether extract (EE)) and waste-based diets on the larval growth and chemical composition was evaluated. Three experimental diets, mainly composed by feed and breading waste (FW and BW, respectively), were formulated as follows: (TM1) FW and BW + wheat groats, wafer dough, vinasse and silvery film, (TM2) FW and BW + wafer dough and panettone waste and (TM3) FW + BW + rice by-products. A wheat bran control diet (C) was also set-up. The trial started with TML of 3 weeks old (WO) of age (5 replicates/treatment; 10,000 larvae/replicate). During the trial, larvae were weighted every week (samples of at least 100 larvae/sample). At the end of the trial, the biomass and frass were weighed, and the total number of larvae was estimated. After freeze-drying, larvae chemical composition (dry matter (DM), CP, EE, ash and chitin) was assessed. One-way ANOVA (SPSS, v28.0) was used to analyze data. The survival rate was not affected by the dietary treatment. As concern growth, no differences were recorded until 6 WO, while at 7 WO TM1 larvae reported the best growth ($P < 0.05$), followed by TM2-3, which performed similarly, and C larvae reported the lowest values. ($P < 0.05$). At 8 and 9 WO, TM3 and C larvae were the heaviest and lightest ($P < 0.05$), respectively. At 10 WO, all treatments were statistically different (TM3 > TM2 > TM1 > C; $P < 0.05$). As far as the chemical composition is concerned, differences were reported among diets, with C larvae showing the lowest DM and EE, as well as the highest CP and ash content ($P < 0.05$). The experimental diets composed mixing waste, showed similar DM, EE and CP. In short, the dietary treatment affected the growth performance of the larvae, also if diets were iso-nutrient. TM3 diet, which contained rice by-products, displayed the best result.

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Mediterranean agricultural side-streams for insect rearing: the CIPROMED approach

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The valorisation of agricultural side-streams through insect bioconversion can aid Mediterranean countries in leveraging locally available resources for animal feed production from insect biomass, thereby enhancing the resilience of farming systems in the region. Within the CIPROMED project, a diverse range of agricultural side-streams generated in Greece, Tunisia, Israel and Germany were identified, gathered, and biochemically analysed. This initial phase aimed at identifying locally available side-streams for use as dietary ingredients for the most commonly raised edible insect species, namely the black soldier fly, *Hermetia illucens*, and the yellow mealworm, *Tenebrio molitor*. Specifically, brewer's spent grains and yeast, oilseed press cakes (from sunflower, line, cannabis, thistle or pumpkin), food processing residues (whey milk, waste milk, biscuits, potato waste, expired yogurt and eggshells grinded) and agricultural by-products (olive pomace, dates, date seeds grinded, cereal milling waste, grape pomace, maize gluten and wheat gluten) were collected and subjected to analysis. The results indicated significant variability in the nutritional composition of the selected side-streams. For instance, their protein content ranged from 3.6 to 89.2%, while their fat content varied between 0.4 and 58.6%. Likewise, diverse results were observed for their dry matter (5.7 to 98.9%) and carbohydrate content (0 to 81.5%). In the subsequent phase, side-stream-based diets will be formulated and assessed at laboratory and pilot scale for the rearing of *H. illucens* and *T. molitor* larvae based on the composition of the chosen side-streams and the nutritional requirements of the insects. Financial support for this research has been provided by PRIMA, a program supported by the European Union (CIPROMED, Grant agreement No 2231).

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Enzymatically treated lignocellulosic feedstock as a source of feed for yellow mealworm – long term results

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The yellow mealworm (YM) is a species perceived as one of the most suitable for large-scale insect farming. The feed used in its rearing are usually dry residues from the agricultural and food industry containing protein and dietary carbohydrates, such as cereal brans, wet and dry distillers grains, second grade grain from cleaning, oil press cakes and meals. Multiple studies have shown that the use of residues containing significant amounts of structural carbohydrates, such as cellulose or hemicellulose, as feed for YM is not suitable. Therefore, in our research we tried to liberate easily available sugars from lignocellulosic materials, for the purpose of insect rearing. Two lignocellulosic substrates (wheat straw and cup plant) where used as substrate for feed material. They were pretreated with steam explosion, the organosolv process, enzymatic hydrolysis and their combination and then used as a sole feed or mixed with wheat bran (also used as control). In general, the more lignocellulosic materials the less was the insect weight and less survival. Insects reared on sole lignocellulosic feed did not grow at all, and the mortality rate was as high as 99%. However, after mixing the above-mentioned substrates with bran the individual larva weight was from 80 to 130 mg and survival reached from 70 to 90%. In conclusion, the addition of various lignocellulosic substrates resulted in poorer development, survival and feed conversion efficiency of yellow mealworm, especially in the case of its high proportion in the diet. Acknowledgement These results are a part of research project entitled “Growth performance, chemical composition and valorisation of residues of yellow mealworm fed with pretreated lignocellulosic biomasses“ (2GenBug) funded by National Science Centre, Poland, grant no. UMO-2020/39/I/NZ9/00907.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

The potential of using Nordic shrimp powder as a finisher-feed in black soldier fly (*Hermetia illucens*, Linnaeus, 1758)

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As food security issues increase from the global growth population in the current climate crisis, organic waste management by larval bioconversion of the black soldier fly (BSF) allows the valorization of local agricultural coproduct. In the North Atlantic fisheries industry, shrimp shells and heads are transformed into a safe and edible powder with high contents in PUFAs and chitin. It was hypothesized that an increase of PUFAs in the diet could improve the quality of the larval fatty acid composition and thus modify pupation rate and enhance reproductive success (clutch number, egg mass, emergence, sex ratio, fecundity). However, hydrolysis of chitin complexes should be also performed to increase the protein digestibility of the powder. This preliminary project involved nutritional trials (n = 3/diet; n = 600 larvae/tank; 30 mg/ind/d) using a finisher-feed approach at prepupal stage (day 10 to 17 post-hatching) with (10% inclusion, dry basis) or without (Gainesville diet, 70% HR) crude or hydrolyzed shrimp powders. No significant difference in growth performance or bioconversion was observed. However, the imago's rate of emergence in rearing cages (28 ± 14 vs 18 ± 8 vs 18 ± 5 flies/d) decreased while the hatching of neonates remained stable in supplemented larvae. These preliminary results will provide a better understanding of the advantages and limitations of including shrimp co-product substrate in fly production as well as the technical and economic feasibility to offer new local markets for shrimp processors.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Yellow mealworm rearing and processing in Poland

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Insects are known for their efficiency in converting various sources of biomass into a rich source of protein and nutrients. The yellow mealworm (YM) (*Tenebrio molitor* L.) is one of the most popular farmed species and has the status of a farmed animal in EU. The larvae of this species are rich in protein, fat, vitamins and minerals and can be successfully used as a source of feed and food. This presentation contains the results of research on the use of biomass residues in YM larvae rearing and processing conducted in 2019-2024 in our department. The aim of our studies was to utilize various biomasses of agricultural and industrial origin in YM rearing as feed and as water sources. The studies, conducted both on a small and larger scale, used commercially available residues (e.g. wheat and rye bran, cakes and meals from oilseed pressing). The research determined, i.e. growth and survival parameters of larvae, feed use efficiency, and composition of YM (e.g. proximate analysis, fatty acid composition). The results revealed that survival of YM larvae was high and varied between 92.2% and 97.7%. Larvae development time last 73-115 days depending on the diet. The results revealed the lowest FCR (1.53-1.59) were found on larvae fed on rapeseed meal mixed with chicken feed. Our preliminary research also showed that although YM larvae can develop without an additional source of water on dry feed, the lack of it slows down their growth by 2.5 times. Protein and fat content in YM larvae ranged from 43.6 to 53.4% d.m. and from 22.3 to 30.0% d.m., respectively. The major fatty acids in all YM samples were oleic acid (32.97-46.74% of total fatty acids (TFA)), linoleic acid (22.79-38.98% of TFA), and palmitic acid (12.80-17.81% of TFA), except larvae fed with mixture of wheat bran and flax cake, where α-linolenic acid content increased 17 times. The research was financed by the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering (grant No. 30.610.007-110) and by the Minister of Science under ”the Regional Initiative of Excellence Program”.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Identification of potential volatiles as indicators of stress by black soldier fly larvae

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There is a clear need to develop diagnostics to ensure optimal conditions while minimizing stress as related to animal welfare with the black soldier fly (*Hermetia illucens* (L.) (Diptera: Stratiomyidae). For ensuring the well-being of traditionally farmed animal, the application of the "5 freedom of Brambell Report" is in use. The animal should be free from hunger and thirst (1); discomfort (2); pain, injury, and disease (3); to express normal behavior (4); from fear and distress (5). It is important to develop tailored techniques for appropriate stress evaluation in insects, managing them as sentient creatures. Volatile organic compounds (VOCs) released by insects could serve such a purpose. This research focused on black soldier fly larvae (BSFL) given their global value to the insects as feed industry. The objective of the current study was to examine VOC emissions from larvae after harvest and during a 24 h starvation (i.e. finishing step) period under low and high stress conditions. This finishing step allows the larvae to evacuate their gut prior to the 'kill step' but unfortunately, it can often be performed under conditions that may cause them stress. We focused on VOC emissions during the finishing step under different light (e.g. light v no light) and temperature conditions (25 °C vs 40 °C). As for farmed animal stress is defined as "reflex reaction that occurs when animals are exposed to negative environmental conditions, causing unfavorable consequences, from discomfort to death", any changes in insects' VOCs emission in terms of quantity and quality of compounds reflect their biological response to challenging parameters. Identification of indicator volatiles could be developed into real-time methods for monitoring BSFL for stress responses. When detected at a threshold indicating stress, system adjustments can quickly be made to alleviate stress while increasing survival and larval chemical quality, optimizing their final use in the feed sector.

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Insect frass enhances yield and improves product quality in a Greek variety of pepper

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The use of insect frass, which is a byproduct of insect farming, as a soil improver and fertilizer has recently gained considerable scientific and commercial attention, due to the encouraging results concerning its effectiveness in plant growth promotion. This study evaluates the efficacy of frass in optimizing pepper yield and quality compared to commercial chemical fertilizers, as well as poultry manure, a widely used organic fertilizer. The Greek pepper variety "Florinis" was cultivated for three months in pots filled with either medium-to-heavy or sandy soil under 6 treatments, i.e. a negative control (only water), a positive control (chemical inputs), 0.5% and 1% w/v frass and 2.5% and 5% w/v poultry manure. The impacts on yield parameters (number of peppers per plant and total production per plant), and qualitative parameters (colour, flesh hardness, total sugars, and pH) were assessed. The results indicated that 1% frass and 2.5% poultry manure amendments both significantly increased all measured yield and quality characteristics in both soil types, compared to the other treatments. Indicatively, in the medium textured soil, the 1% frass treatment increased total fresh pepper biomass by 50.5% over the negative control and 34.1% over the positive control. In the sandy soil, the corresponding increases were 81.1% and 62%. The optimizing effect of frass and poultry manure on all parameters assessed was more pronounced on the infertile sandy soil compared to the medium textured soil. This study highlights the potential of frass and poultry manure as effective organic fertilizers for improving both pepper productivity and the quality of the final produce. This research is supported by the EU-PRIMA program project ADVAGROMED (Prima 2021 – Section 2).

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Varolization of agri-food side-streams as dry and wet feed of the yellow mealworm, *Tenebrio molitor*

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The agri-food sector generates a huge amount of side-streams that could be upcycled through their bioconversion by edible insects into valuable nutritive sources. Regarding the yellow mealworm, *Tenebrio molitor*, recent research has mostly focused on dry waste streams provided to the mealworms as dry feed. Apart from dry feed though, moisture availability during rearing of larvae of the yellow mealworm is critical and can greatly enhance larval growth performance and development. However, published works on the evaluation of side-streams as wet feed are limited. Oilseed presscakes, the solid residues of oil extraction from oilseeds that still contain some valuable nutrients, have not been thoroughly evaluated for *T. molitor* rearing. Similarly, milk whey, either acid whey (AW) or sweet whey (SW), is one of the primary side-streams of the dairy sector, totaling several million tons annually. In this context, the objective of the present study was to evaluate oilseed presscakes and milk whey as dry and wet feeds of *T. molitor* larvae, respectively. Briefly, in a first series of laboratory bioassays oilseed presscakes from sunflower, line, cannabis, thistle, and pumpkin were evaluated as larval feeding substrates. Additionally, in a second laboratory bioassay, AW and SW were evaluated as larval wet feeds. Agar, commonly used in artificial diets, was used as gelling agent to produce whey-based agar gels that were provided to the mealworms as a moisture source. The results of the present study showed the potential of both oilseed presscakes and milk whey to be utilized as dry and wet feeds for *T. molitor* larvae. This work aims to contribute to the integration of side-streams of the agri-food sector with mealworm farming, as part of a circular economy that will enhance the sustainable profile and reduce the environmental impact of mealworm production. Financial support for this research has been provided by PRIMA, a program supported by the European Union, under grant agreement No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

INSECT DIETS, NUTRITION AND PHYSIOLOGY

Temperature conditions for long-term preservation of black soldier fly strains

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As the black soldier fly (BSF; *Helmetia illucens*) lacks diapause ability, BSF strains must be reared continuously throughout the year. This is problematic for BSF breeders in the laboratory because living stocks increase during the crossing and selection of potential strain sources. BSF production facilities also need technology to adjust breeding schedules depending on the availability of diet and environmental conditions. This study developed methods for the long-term preservation of living BSF strains. Control BSF were reared throughout the life cycle at 28 °C. In experiment 1, day-7 larvae reared at 28 °C were moved into an incubator and kept at 16 °C for 70 days to suppress growth. The larvae were reared at 28 °C again to adult stages. In experiment 2, day-7 larvae reared at 28 °C were moved into an incubator and kept at 16 °C for 70 days, as in experiment 1. The larvae were reared at 28 °C to prepupae stages. The pre-pupae were moved into an incubator and kept at 10 °C for another 70 days to extend the lifecycle. Then, the pre-pupae were reared at 28 °C to adult stages. The temperatures and growth stages suitable to suppress growth were decided from preliminary studies. The average lifecycle of control BSF was 50 days from egg to adult. In experiment 1, the average lifecycle was increased to 118 days. The pupation rate was 74.8%, and adults emerged from all pupae. In experiment 2, it was further increased to 185 days, and eclosion rate was decreased to 66.7%. The weight of eggs per adult decreased in the order of control (1.49 mg) > experiment 1 (0.74 mg) > experiment 2 (0.18 mg). In our study, storing black soldier fly (BSF) larvae at 16 °C and prepupae at 10 °C can extend the lifecycle to >180 days. This technique is useful in preserving many stocks of BSF strains in the laboratory or adjusting the life stages in breeding facilities. The lifecycle can be extended by adjusting duration, but attention should be paid to the trade-off between prolonged exposure and reduced reproduction.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Physical waste properties alter temperature, respiration and rearing performance in black soldier fly bioconversion

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Challenges in recycling of qualitatively ever-changing biowaste with black soldier fly larvae can be elevated heat production and low/variable process efficiency. Heat production is poorly understood but can result in larval escape and overdrying of the biowaste for larval digestion and harvest. In this study, the influence of biowaste bulk density (BD) was assessed which is via O_2 availability related to biowaste temperature, overall respiration, substrate respiration and bioconversion metrics. The study involved a controlled feeding experiment with industrial rearing crates (7 days, 10,800 larvae, 25 mg dry biowaste × larva × day) in three parallel respiration chambers. Different physical conditions in the biowaste (canteen leftovers) were created with a bulking agent (coconut fiber, high: 762 kg m^{-3}, medium: 637 kg m^{-3}, low 508 kg m^{-3}). Biowaste temperature was as well as larval and microbial CO_2 production from each respiration chamber (hereafter referred to as respiration) were sampled. To complement, dry larval mass and microbial abundance (using qPCR) were measured every second day. BD influenced dramatically rearing performance metrics, temperature, and respiration. Particularly, until rearing day 2, biowaste temperature, respiration and larval weight were higher the lower the biowaste BD. Until rearing day 4 biowaste temperatures stayed below 35 °C under high BD, above 40 °C under low BD, and ranged in between beforementioned under medium BD. Final larval mass was 81% and 52% higher under high BD and medium BD biowaste respectively compared low BD biowaste. It suggests that low BD biowaste likely enables more O_2 supply for microbial respiration resulting in microbial heat production. The nutrients consumed for this heat generation could have constrained the final larval mass in biowaste with lower BD. This study contributes to the understanding of temperature dynamics in black soldier fly larvae bioconversion and suggests that heat production can be (partially) manipulated by physical properties of biowaste.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Bioaccumulation of phytochemicals and antioxidant activities of *Hermetia illucens* larvae raised on different substrates

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Limited studies have reported on the bioaccumulation of phytochemical and antioxidant properties in black soldier fly larvae (BSFL) raised on food waste. In this study, bioaccumulation of phytochemical content and antioxidant activity in BSFL raised, firstly on different concentrations of fruit and vegetable (FV) waste and secondly on food industry (FV, grape marc (GM), olive pomace (OP)) waste were investigated. BSFL neonates were fed different food substrates from day 10 until maturity. Protein and fat fractions of BSFL were analyzed for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities using ferric reducing antioxidant potential (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), and oxygen radical absorbance capacity (ORAC). Protein fractions of BSFL fed 85%FV showed a significantly higher TPC (340.94 ± 6.64 vs 265.78 ± 14.67 µg GAEq/100 mg, $P = 0.01$) compared to 50%FV. Similarly, BSFL fed on 85%FV showed a significantly higher TFC in protein (194.68 ± 13.95 µg QEq/100mg, $P = 0.01$) and fat fractions (213.36 ± 5.33 µg QEq/100 mg, $P < 0.01$), when compared with 50%FV. ORAC was significantly higher in protein fractions of 85%FV (25072.30 ± 48.75 vs 7995.24 ± 267.86 µmol TEq/100 mg, $P < 0.01$) when compared to 50%FV. When comparing the food industry waste, BSFL raised on OP substrate showed a significantly higher ($P < 0.05$) TPC (protein: 364.93 ± 3.49 µg GAEq/100 mg) and TFC (protein: 185.14 ± 29.42 µg QEq/100 mg and fat: 169.42 ± 10.57 µg QEq/100 mg) when compared to FV and GM. ORAC levels were significantly higher in protein fractions of BSFL fed GM substrate (21970.11 ± 14.67 µmol TEq/100 mg) when compared with FV and OP. Both protein and fat fractions of BSFL raised on FV substrates showed significantly higher DPPH and ABTS levels when compared with other substrates. The feed present in the GI tract of the larvae could be the main trigger for differences in effects.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Ammonia and GHG emission during organic waste conversion by using black soldier fly larvae

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Using black soldier fly larvae (BSFL) to convert organic waste has become a research hotspot. However, there are few studies on the characteristics of gas emission during the conversion process and the subsequent composting of residue (frass). Effects of 12 different conditions on gas emissions, larvae growth and residual moisture were evaluated with a production-scale. NH_3 and GHG emissions during the whole conversion process were daily recorded. Strategies of multiple-stage feeding and enhancement of air supply were applied to reduce NH_3 emission. The frass of BSFL conversion and chicken manure were further used for aerobic composting, respectively. Results and discussion: The emission of NH_3 caused 1.87% to12.11% of total nitrogen loss in different conditions during food waste conversion, while the emissions of CH_4 and N_2O was at a level around 15.43 mg/kg and 6.85 mg/kg. The main factor affecting NH_3 emission is the moisture content of food waste. Adding hollow plastic ball reduced NH_3 emission by 44.37%. When fed with chicken manure, the emission of NH_3 was 1,046 mg/kg during BSFL conversion (147 mg/kg) and subsequent frass composting (899 mg/kg), while aerobic composting generated 1,999 mg/kg. The CH_4 emission of BSFL conversion reached 42.90 mg/kg, which was significantly higher than aerobic composting (0.55 mg/kg). The N_2O emission of BSFL conversion was only 0.42 mg/kg, which was about 2% of that during aerobic composting. The feed property and breeding conditions significantly affect NH_3 emission during BSFL conversion and the main factor affecting NH_3 emission is the moisture content. Compared with aerobic composting, bioconversion of organic wastes with BSFL reduced 47.63% NH_3 emission and 98% N_2O emission, but with higher CH_4 emission, which indicated that using BSFL to convert organic waste is a more environmentally friendly strategy.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Ultrasonic pre-treatment of lawn clippings as feed for black soldier fly larvae

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Black soldier fly larvae (BSFL), *Hermetia illucens*, efficiently decompose organic waste. However, substrates like grass and leaves contain lignocellulosic components in their cell walls, restricting BSFL's access to sugar in cellulose. Pre-treating by ultrasonication could enhance the availability of nutrients by breaking lignin and breaking down cellulose and was thus tested for its impact on BSF larval performance. As this pre-treatment might affect the microbial community in the substrate, the pre-treated material, was supplemented with different inoculants. Freshly cut grass from public parks was shredded (meat grinder and kitchen blender) and diluted to DM content of 6%. Pre-treatment involved a laboratory ultrasonic processor (Hielscher UP400St 400W) set at 100% amplitude for 19 hours. Substrate samples, treated and untreated, were spiked with one of four inoculants: fresh grass cuttings, BSF frass, dead BSF adults, and probiotics (enterococci strain SF 68). Moisture content of substrate was 90%. Larvae were fed 40 mg volatile solids/larva/day for 14 days (feedings on day 0, 3, and 9). Feeding experiments (triplicates) used plastic containers (Ø7.5 cm, 11 cm height) with 30 five day old larvae. Neither ultrasonic pre-treatment nor the different inoculants had a significant effect on the dry weight of the harvested larvae. The larval weight ranged from 72.3 mg dry weight (no pre-treatment, probiotics) to 87.3 mg dry weight (ultrasound, dead flies). Substrate reduction was highest in both untreated and pre-treated samples when grass cuttings (36.7%/34.1%) or dead flies (33.5%/36.4%) were added. The least impact (28.2%/20.5%) was observed in when frass was added as inoculant. While our experiment did not reveal immediate advantages of ultrasonic pre-treatment, the remarkable larval growth using lawn clippings as the sole feed suggests the potential for BSFL to efficiently manage municipal green waste. These findings could significantly expand the utility of BSFL for treating municipal green waste, offering an economically attractive waste management option.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Quantifying co-benefits in climate, soil, and economy via on-site composting and urban insect-, fungi-based technologies

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The Canadian food system – responsible for 13% of national greenhouse gas emissions (GHG) and substantial waste – can benefit from circular agrifood systems like Urban Agriculture (UA). Drawing data from a Montreal-based UA cluster, this study evaluates the potential climate, soil, and economic benefits of implementing a circular agrifood system on a national scale. Forecasts suggest a reduction in organic waste (OW) landfill disposal (from 56% to 20%), alongside an increase in on-site composting (from 44% to 60%) by 2035. This paper explores a scenario in which 20% of OW is instead managed via on-site composting (10%), fungi (5%), and insect (5%) farming. Utilizing site-specific data, Life Cycle Analysis (LCA) models highlight a net carbon-negative footprint for managing one tonne of OW through fungi and insect farming (–16.5 kg and –160 kg CO_2e/tonne, respectively). This underscores the substantial reduction in GHG achievable through insect farming. Scaled up to Québec, these UA technologies can divert 1,141 kilo tonnes OW annually by 2035, reducing provincial GHGs by 27%. On a national scale, that could generate $8.9 billion in annual revenues and employ 18,282 individuals by 2035. Despite these positive findings, our model has shortcomings and assumptions. Future research should refine the LCA model, include empirical measurements of on-site GHG fluxes and soil health, and incorporate system dynamics models. Combining modeling improvements with fundamental research on microbiology and genomics of emerging biological OW treatment will provide much-needed insights into the potential co-benefits of these nature-based technologies.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Using mealworms to improve circularity of horticultural production in New Zealand

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New Zealand produces a range of horticultural products, from fresh fruit to wine and nutritional extracts. While improvements are being made in orchards to improve sustainability of production (e.g. returning thinned fruit and pruned plant material to the soil), some waste is still produced. This is particularly the case in winemaking, where a sustainable solution for the 50,000 tonnes of grape marc and wine lees that are produced annually is lacking. Similarly, other horticultural and food processing waste products are currently sent to landfill. To improve this situation, we tested a range of insect species for their ability to feed on grape marc. Mealworms (*Tenebrio molitor*) were the most successful, and we found that 1 kg of mealworm larvae could consume 2 kg of grape marc in 7-10 days. Weight gain of mealworms fed a mixture of marc and wholemeal flour at a ratio of 3:1 grew at the same rate as those fed flour alone. Since mealworms can also feed on other fruit and vegetable waste as well as dry product waste (e.g. from bakeries), we are investigating mealworms as bioconverters of a variety of New Zealand waste streams. We present our results from testing the ability of mealworms to convert a variety of horticultural products into insect meal and frass. Ideally, frass and other insect waste will be able to be used as soil amendments on land used to produce the horticultural products. In time, it may be possible to develop regionally based insect solutions for the waste streams that vary across New Zealand.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Valorization of fermented hatchery residues by black soldier fly larvae (*Hermetia illucens*)

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The valorization of hatchery residues (unmarketable chicks and eggs; HR) by black soldier fly larvae (BSFL) could offer a sustainable alternative to heat treatment rendering. Fermented HR constitute energy-rich diets with reduced microbiological risk. It was hypothesized that BSFL could efficiently bioconvert fermented HR without affecting the quality of the resulting larvae. Control (Gainesville), unfermented HR and fermented HR diets (14 days semi-anaerobic fermentation; 0.3% Lacult-SAX-01 starter culture; 25% lactose; dry basis) with or without the addition of potato residues (13%, dry basis) were fed to BSFL over three trials. A total of 800 5-day-old BSFL were inoculated into each diet in triplicate (0.6 g diet/ind.; dry basis). Microbiological analyses were conducted on initial diets, as on larvae at harvest. The total yield and bioconversion rate for unfermented (60 ± 7 g; 5%) and fermented HR diets (19 ± 4 g; 2%) were significantly lower than those for the control diets (209 ± 18 g; 14%). Fermentation effectively reduced the initial coliform loads (<2 log cfu/g) of the HR to values below the control diet (4 log cfu/g) and unfermented HR (5 log cfu/g). However, fermentation did not provide effective spore control, resulting in high *Clostridium* spp. loads in these diets (8 log cfu/g) and in the resulting larvae (9 log cfu/g). Even with the control diet, the resulting frass and larvae were significantly more contaminated with *Salmonella* spp. (4 log cfu/g) and *Listeria monocytogenes* (4 to 5 log cfu/g) than those from unfermented HR diets (3 log cfu/g). This project demonstrated that HR can be utilized for rearing BSFL, but diet composition and physical characteristics will need optimization to promote bioconversion. Although the microbiological quality of the initial diet can be modulated by its composition and fermentation, subsequent treatments of larvae are required to ensure microbial safety.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

The potential of black soldier fly farming in Malawi: challenges and opportunities

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Waste management is still a big challenge for the Malawi government. Out of the waste generated of which over 80% is organic waste, only 30% is collected and disposed of at landfills. Organic waste if not effectively managed contributes to greenhouse gas emissions and the loading of nutrients in water bodies. However, for countries like Malawi that are struggling with food insecurity due to poor and degraded soils, effects of climate (e.g. droughts) and the rising cost of chemical fertiliser, organic waste has the potential to lessen the situation. Technologies exist that can be used to recycle organic waste into a resource to be used in agriculture. However, recently, the use of black soldier fly (BSF) has gained momentum and farmers in Malawi are adopting the technology. In addition to producing frass for crop production, BSF contributes to the animal feed (poultry, fish, and pigs) industry through the larvae. BSF farming is new in Malawi and lacks the proper infrastructure to support the current and would-be farmers. Therefore, this study was designed to map the BSF farmers in Malawi and to find out the challenges they are facing. The results showed that BSF farmers are spread across the country with the majority in Lilongwe and Blantyre. Only 35% of the farmers had BSF colonies, the rest were just willing to start farming BSF. Most farmers (70%) were interested in BSF to produce animal feed. As farmers were keeping BSF and were willing to keep, lack of knowledge and experience, lack of capital, scarcity of construction materials, lack of larvae markets, and waste purity were some of the challenges hampering BSF farming in Malawi. It is clear that BSF farming has immense potential in Malawi, but support is needed for BSF to thrive in Malawi. Policies and regulation frameworks should accommodate BSF farming and that will unlock support for BSF farming in Malawi.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Pretreatments to enhance black soldier fly larvae bioconversion of fibrous biowastes

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Black soldier fly larvae (BSFL, *Hermetia illucens* (L.)) presents a promising opportunity for biowaste management, transforming biowastes into a protein-rich animal feed and nutrient-rich fertilizer. However, their efficiency in processing low-value biowastes that contain high lignocellulosic fibres is limited, typically resulting in slower larval development and reduced growth. Given the limited research on the effects of biowaste pretreatments for BSFL performance, this study addressed the gap by reviewing the applicability of pretreatment methods from other bioprocessing technologies. Based on the review, an assessment was conducted on the impact of chemical, thermal, and mechanical pretreatment in enhancing BSFL bioconversion of three fibrous biowastes: cow manure, spent grain, and grass clippings. Larval feeding experiments were performed in a climate-controlled chamber (9 d, 28 °C, 44-70% relative humidity, 35 mg dry mass (DM)/larva/day, 2.5 larvae/cm^2, n = 4) on both pretreated and untreated substrates. Aqueous ammonia pretreatment for 3 days reduced BSFL rearing performance metrics by over 50% compared to the untreated controls and revealed a dose-dependent toxicity with ammonia pretreatment. Thermal pretreatment at 90 °C for 0.5, 1 and 4 h did not change larval growth on any substrates. Contrarily, mechanical pretreatment, specifically milling of spent grain and grass clippings with screen sizes of 0.5, 1, and 2 mm, demonstrated improvements. For example, larvae grown on 0.5 mm-milled spent grain and grass clippings increased larval DM by 15-19%, accompanied by a 23-41% increase in larval protein conversion compared to untreated. This study found mechanical pretreatment had the best potential in enhancing BSFL bioconversion of fibrous biowastes, offering valuable insights for the advancement of biowaste management and enhancement of protein production.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Impact of the size and shape of microplastics on their ingestion and excretion by BSF larvae during waste bioconversion

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Black soldier fly larvae (BSFL, *Hermetia illucens*) can valorize food waste into high-value products, including animal feed. However, these food wastes may contain microplastics originating from food packaging eventually compromising the growth and the safety of larvae as feed. This study investigates the impact of polyethylene microplastics size and shape on the growth of BSFL and on the ingestion and egestion kinetics during waste bioconversion. BSFL were reared on artificial food waste containing fruits, vegetables, bread, and dairy products (25% dry matter, single batch of 100 mg/larvae/day) and spiked with spherical or irregular self-produced fluorescently labelled microplastics (26,000 particles/g of substrate). Three size ranges were tested in triplicate for each shape (from 38 to 150 µm) and compared to controls. Throughout the experiment, the larval growth performance was determined and the amount of microplastics in the larval gut was monitored daily (n = 9 per treatment), using fluorescence microscopy. We observed that the presence of microplastics had no impact on larval growth, having a mean maximal larval weight of 221.4 ± 23.3 mg. Further, no bioaccumulation of microplastics (BAF < 0.3 ± 0.1) was observed. However, significant differences in microplastic ingestion were observed based on their size. While a steady increase in microplastics was observed in the larval gut for the smaller particles, the larvae exhibited minimal ingestion of particles surpassing 100 µm. Furthermore, kinetic modelling of the temporal microplastics ingestion and egestion dynamics showed that particle shape can slightly modify the kinetic parameters of ingestion without having any distinct effect on the bioaccumulation. Finally, a 3-day starvation period appeared to be effective in reducing the number of microplastics present in the larval gut by 95.9 ± 4.0 %, but the associated larval weight reduction (30.5 ± 6.6 %) is unfavorable for industrial applications.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Efficiency of rearing black soldier fly larvae (*Hermetia illucens* L.) on different industrial and domestic waste sludges

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Black soldier fly larvae (BSFL) are recognized for their exceptional ability to valorize diverse waste streams, efficiently converting organic waste into valuable products. This study aimed to evaluate the efficiency of rearing BSFL on various industrial and domestic waste sludges to include them in a circular food system. Thirteen sludges of diverse origins were tested in a small-scale experiment. Among these sludges, BSFL reared on six sludges exhibited superior growth performance in terms of survivability and growth rate. The six selected sludges, namely potato processing sludge (PS), dairy sludge (DS), sugar processing sludge (SS) and three domestic sludges from distinct locations (small city SCS, big city BCS and island IS), were further evaluated in triplicates in a large-scale experiment, with chicken feed (CF) as a control. The crude protein content of the substrates ranged from 18.5% (CF) to 53.5% (PS), and the crude fat content varied between 1.2% (SS) and 5.3% (BCS). Various parameters including larval growth performance (larval weight and growth rate), survival rate, waste reduction index (WRI), conversion efficiency (ECI), and larval biochemical composition were analysed. Frass biochemical composition was also analysed. Among the tested substrates, DS-reared larvae had the highest growth rate (8.9 mg/day) and WRI (7.9 g/d), and PS-reared larvae had the highest larval survival rate (87.1%) and ECI (0.3) compared to other substrates. Conversely, larvae reared on SCS and SS showed inferior performance compared to other substrates, with the lowest growth rate (1.9 and 2.3 mg/d, respectively), survival rate (60.7% and 59.2%, respectively) and ECI (0.08 and 0.09, respectively). The WRI was the lowest (4.1 g/d) when larvae were reared on SS. In conclusion, this study showed that the selected sludges can serve as a nutritious component of the substrate to grow BSFL, showcasing their potential in sustainable waste management and circular food systems.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Small-scale optimization of probiotic treatments used in black soldier fly digestion of dairy manure

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Black soldier fly larvae (BSFL), *Hermetia illucens* L., can digest a variety of organic residuals. Because of this, they are mass reared on such materials to produce protein and other materials, such as fertilizers and biodiesel production. However, not all byproducts yield the highest larval quality. In the case of dairy manure, BSFL can digest the material; however, the larvae produced are not as heavy or do not reduce the organic matter as much as those reared on a control diet. Research has shown that when a Gainesville diet is supplemented with probiotic treatments, larvae increased in weight and had faster development time versus those fed a diet without the probiotic; however, our preliminary work with supplementation in dairy manure at the industrial scale did not show a significant effect of treatment leading to the need for optimization of this protocol. The goal of this work was to optimize the concentration of probiotic treatments in dairy manure for the production of BSFL. Because dairy manure is fibrous and some bacteria have cellulase activity, we used a probiotic pre-treatment of dairy manure for one and three days with *Arthrobacter* AK19, *Rhodococcus rhodochrous* 21198, and a combination of both probiotics before adding BSF larvae to allow some of the fiber to be broken down. We also wanted to examine the effect probiotic concentration on larval weight and bioconversion, so we used treatments at 10^6 colony forming units (CFU) and 10^9 CFU. Daily weight of larvae was recorded until a decrease for three days was determined to ensure peak growth was achieved; at which point, larval production and bioconversion were determined. Based on previous work, we expect to see an increase in larval weight and bioconversion rate for the higher concentrations and longer pre-treatment times. While optimization occurs at a benchtop scale to utilize resources effectively, these results can be validated at larger industrial scale to more effectively utilize BSFL in the digestion and waste reduction of dairy manure.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Valorization of organic waste streams: black soldier fly bioconversion performance and contaminant accumulation

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The utilization and experience with various organic streams as feedstock for black soldier fly (*Hermetia illucens*) rearing remain limited in EU countries due to strict legislation. This study aimed to investigate the impact of the inorganic (metals) and organic pollutants (veterinary antibiotics) in different types of by-products and waste streams on the growth performance and potential bioaccumulation in black soldier fly larvae. A series of experiments under realistic scenarios of contamination with metals and/or veterinary antibiotics (manures) revealed the larvae's capability to bioaccumulate these substances. Metal accumulation potential in larval tissue varied among different by-products: Cd (0.7 μg/g for rice, 0.16 μg/g for oatmeal, 0.2 μg/g for bread), Zn (46 μg/g for bread), Mn (118 μg/g for brewery spent grain, 110 μg/g for rice, 35 μg/g for rice), and Fe (66 μg/g for brewery spent grains, 26 μg/g for rice). The reported accumulation of Cd in the insect could be the most problematic phenomenon, particularly when comparing the results with the most stringent limit of cadmium concentration at 0.05 μg/g, as specified by Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food. On the other hand, according to Directive 2002/32/EC on undesirable substances in animal feed, the levels of the substances in BSF larvae met the limits for livestock feed. The second experiment demonstrated the bioaccumulation of veterinary antibiotics from manure, dependent on the initial concentrations. No bioaccumulation was observed at 1 μg/g of antibiotic concentration in manure, but an increase to 10 μg/g resulted in a bioaccumulation of 744.4 ng/g. Considering the use of BSF larvae as part of an animal feed diet, the understanding the dynamics of commonly present pollutants within this process is crucial to ensure process safety and efficacy.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Role of functional microbes in a whole industrial chain of organic waste conversion by black soldier fly larvae

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Hermetia illucens L. (Diptera: Stratiomyidae), also known as black soldier fly (BSF), can be found in decaying organic wastes, such as animal manure and food waste. Over the past several years, investigators in our group have made some research and application progress on BSF breeding and organic waste conversion by BSF larvae and microbes. We isolated some microbes that could induce BSF adults to lay eggs and used in large scale BSF breeding factory. It allows the high efficiency eggs mass production of BSF larvae to meet demand of large-scale organic waste conversion at low cost. Microbes from BSF also play important roles in BSF larvae nutrition and resistance against pathogens. BSF larvae gut microbiota also could help reduce the risk of antibiotics and resistance genes in animal manure. Bacteria isolated from the BSF larvae gut and eggs can be cultured and used to promote BSF growth, and shorten the time to the prepupal stage in lab and in factory. These processes greatly reduce environmental impact caused by organic waste. Application of BSF larvae and microbes for recycling animal manure is well established. The residues collected from BSF larvae conversion are subjected to secondary fermentation using nematocidal microbes to produce functional organic fertilizers and have good efficiency against root rot nematode in field. The technology combined BSF and microbes could reach low emission and high-value utilization as animal feed.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Unravelling aflatoxin B1 biotransformation by the black soldier fly (*Hermetia illucens* L.)

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Aflatoxins are known contaminants in agricultural crops across the globe, which are toxic to animal and human health and necessitate innovative management strategies. In the current study, we exposed larvae of the black soldier fly (BSFL; *Hermetia illucens*) to diets spiked or naturally contaminated with aflatoxins. The aim was to determine whether BSFL could offer a potential solution for aflatoxin management. In our study, we spiked feed substrates with aflatoxin B1 (AFB1) to a concentration equal to the maximum limit set by the European Commission for feed materials. Additionally, we exposed BSFL to two types of naturally contaminated substrates: peanut press cake and contaminated maize. We analyzed the concentrations of aflatoxins and known metabolites using an LC-MS/MS-based method, examining their presence in the feed, larvae, and residual material. In all cases, survival and growth of BSFL was not affected by the aflatoxin concentrations examined. Furthermore, the BSFL did not accumulate aflatoxins, and the concentrations detected in the larvae remained well below the maximum limit set for feed materials. The molar mass balances of AFB1, determined after spiking, indicated a missing fraction of 38% . Interestingly, the results diverged for naturally contaminated diets, but the formation of the AFB1 metabolite AFP1 remained consistent in all situations. Our findings suggest that aflatoxin metabolism occurred, involving both non-enzymatic and enzymatic degradation pathways for AFB1. Our study reveals that aflatoxin-contaminated substrates, previously deemed unsuitable for livestock, can be used effectively as substrates for rearing insects. These findings present exciting economic prospects in insect-based systems.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Degradation of straw and kitchen waste by black soldier fly combined with pretreatment of cellulose-degrading bacteria

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This study addresses the challenge of low utilization efficiency of agricultural waste, particularly straw due to high cellulose content in straw. To improve utilization, the study proposes microbial fermentation pretreatment to convert cellulose into soluble sugars, facilitating the digestion of straw by black soldier fly larvae (BSFL). Four cellulose-degrading bacterial strains were identified from BSFL gut, chicken manure compost, and agricultural enzyme screening. The strains were evaluated for endoglucanase and xylanase activity. The best performing strain for pretreatment was selected and utilized for further experiments. Sesame straw was pretreated using single strain and a compound strains. The pretreated straw was then mixed with kitchen waste at different ratios to evaluate the performance of BSFL. The results showed Bacillus subtilis RX01 exhibited the highest endoglucanase activity, achieving a 21.9% increase in reducing sugar content after 3 days of fermentation. The compound strains S6 showed superior performance compared to the control and individual strains, with a 39% increase in soluble sugars content. The S6 pretreatment resulted in reduced cellulose and hemicellulose content, increased crystallinity, and altered surface texture of sesame straw residues. The optimal ratio of pretreated straw to kitchen waste (7:3) yielded the highest conversion efficiency and material reduction rate for BSFL. The ratio of straw is from 5% to 30%. In the pilot experiment, we established a 10 kg material conversion system. The pre-treatment group increased the weight gain of water flies by 25.21% compared to the control group, and the biological conversion rate was relatively increased by 28.57%. The study highlights the potential of microbial pretreatment coupled with BSFL. Straw pretreatment with cellulose-degrading bacteria could increase the straw utilization content in straw and kitchen waste BSFL treatment system. Straw pretreatment become cellulose into soluble carbon resource for BSFL conversion.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Use of sequential neural-networks for insect yield prediction based on holistic diet data

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Insect production yield from diverse organic wastes and food by-products is challenging to predict due in part to the heterogeneity of substrate compositions. Heterogeneity of food wastes thus limits their potential for upcycling through insect biotreatment. Recent studies focus on the influence of one or two factors of diet composition on insect bioconversion efficiency (e.g. protein and fat content). This research aims to explore the use of holistic diet data and sequential neural networks to inform predictions of insect production yield. An insect growth dataset was constructed from a series of experiments in climate-controlled conditions using two insect species, *Tenebrio molitor* and *Hermetia illucens*. A total of 20 dietary exposures were tested, consisting of combinations of food byproducts and foodwaste mixtures, each characterized for at least 6 macronutrients, 20 minerals, and 16 amino acids, together with 3 growing parameters (e.g. feed/larvae ratio). Direct observations from these experiments indicated that insect yield was positively correlated with total digestible nutrients and protein content, and negatively correlated with mineral content, and to a lesser extent to C/N ratio and acid detergent fibers. We then incorporated utilize multi-temporal data, including survival and average larvae weight, together with initial diet substrate and final frass composition data in a sequential neural-network. Model parameters and features were tested using different neural-network architectures (e.g. varying the number of dense layers, types of loss and activation functions, iteration and learning rates) to improve prediction efficiency. Application of deep neural networks has the potential to inform the design of efficient insect diet formulations that incorporate a broader range of food byproducts and foodwaste mixtures, and hence, lower the barrier of entry for their direct upcycling through insect processing.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Valorization of banana and winery production wastes using black soldier fly larvae

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Global banana production totalled 125 million metric tonnes in 2021. 60% of the total biomass produced is under-utilized and considered as waste. A further 25% of the utilised biomass is estimated to be out of specification, damaged or overripe fruit, and banana peels and therefore adds to this waste biomass. Global wine grape production surpassed 28 million tonnes in 2023. An estimated 15%, or 4.2 million tonnes, is discarded from the wine making process as organic waste biomass i.e. grape marc (pomace) and lees. Black soldier fly larvae (BSFL) neonates were fed various concentrations of the banana and winemaking by-products. Growth parameters were recorded until larvae reached prepupae stage. Larvae weights and resultant frass were recorded and material reduction and larvae bioconversion rates calculated. These feed trials indicated that: Banana peel and unsaleable fruit are preferred and high growth media for BSFL; banana rachis (flower and fruit stem) are a satisfactory feed when blended with a small quantity of reject fruit. Material reduction ratios of over 80% (by weight) were achieved; grape marc to be a satisfactory feed for BSFL with material reduction ratios of over 60%, and larvae bioconversion rates ranging from over 6% (100% grape marc feed), to over 22% (feed comprising 75% grape marc); pseudo stem can provide a satisfactory feed basis if pretreatment and fermentation is used, and, if feedstock is supplemented with other nutrients. The frass by-products from these bioconversion processes have shown that macronutrients and some micronutrients, are concentrated in the resultant frass. Typically: frass from BSFL fed on banana rachis substrate showed over 57% increase in total nitrogen, 40% increase in total phosphorus, and 13% increase in total potassium; frass from BSFL fed on grape marc substrate showed over 70% increase in total nitrogen, 50% increase in total phosphorus, and 30% increase in total potassium. As cost of primary production pressures continue, farmers and vignerons are seeking localised solutions to waste management and nutrient recovery. Insect bioconversion addresses these challenges.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Advancing circular economy principles through wild/free-range black soldier flies

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In recent years, bioconversion tools, including the black soldier fly (BSF), have emerged as a global, sustainable and innovative solution for biowaste management. Biowaste treatment offers numerous benefits, including the valorization of biowaste into added value, diverse products and functional ingredients to be used in various industries, including food, feed, cosmetics and pharmaceutics. Although extensive research has been conducted on the BSF biowaste treatment system, none have explored the application of circular economy principles in low-income settings using naturally occurring BSF, i.e. free-range populations. This research presents a case study of a localized circular economy (CE) designed to meet the requirements of a peri-urban Tanzanian community through the co-production of larvae and frass. The study highlights the importance of an inclusive BSF system design suitable for a low-income female-run community group. Additionally, the case study provides information on the setup, operation, and performance indices of a free-range BSF production system by comparing the case study design and stakeholder requirements versus the literature. The study also identifies, investigates, and closes the unique resource gap on location, CE-related indicators and their measurements, all while prioritizing participation and social inclusion and promoting innovation in community engagement. By referring to the challenges encountered and future prospects of free-range BSF systems, our case study demonstrates how a community-led free-range BSF system can effectively manage biowaste using circular economy principles and minimal energy and water input in least-developed economies.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Preservation of agri-food byproducts by acidification and fermentation in black soldier fly larvae bioconversion

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The steady year-round provision of feedstock for efficient black soldier fly larvae (BSFL) bioconversion can be challenging, given the variability in the generation of biowastes. To make feedstocks storable for longer periods, this study investigated the effects of three feedstock preservations – wild fermentation, inoculated fermentation, and acidification – for three storage durations (1, 7, and 14 days) on BSFL bioconversion and harvested larval and frass composition. Feedstock preservation was comprehensively characterized by gross nutrients, sugars (monosaccharides and disaccharides), 12 fermentation metabolites (e.g. lactate, acetate, formate, and citrate), and bacterial community analyses (16S rRNA genes). All feedstock preservation and storage durations resulted in a high bioconversion rate (21-25 % dry mass) and larval fresh mass (170-196 mg). Overall, 7-day acidification had the highest bioconversion rates compared to fermented feedstocks. Acidification proved to be most effective in preserving feedstock nutrients, with only a 10% deviation from the initial nutrient content. Fermentation produced typical lactic acid fermentation metabolites with reducing sugar contents; however, adding a lactic acid bacterial inoculum (*Lactobacillus plantarum*, *Enterococcus faecium*) had no benefit, presumably due to the intrinsic high nutrient content and lactic acid bacteria (e.g. 2-7 $\log_{10}$ CFU g feedstock-1). Contrary to expectation, preservations had little influence on Enterobacteriaceae (6.2-7.5 $\log_{10}$ CFU g-1) in freshly harvested larvae. Notably, Salmonella was absent in harvested larvae, independent of the preservation method. Future research should assess the acidification and fermentation of different BSFL feedstocks and investigate the roles of feedstock pH, organic acids, and fermentation metabolites on larval performance. This study advances towards more reliable and efficient insect-based nutrient recovery from agri-food by-products within the food system.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

New life for RIce by-products and agricultural wastes: insects bioconversion for Fish Feed production: newRIFF project

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The current meat and fish production systems are not sustainable anymore, with protein content of animal feed playing a key role. In particular, the consumption of fishmeal and soybean meal is responsible for serious environmental issues (i.e. overfishing, loss of biodiversity, and land overexploitation) and economic concerns due to the constantly increasing price of these commodities. In parallel, the agriculture and agri-food industry generate a considerable amount of organic waste and by-products whose management has an environmental and an economic cost. Insect bioconversion can allow the re-utilization and valorisation of these by-products to produce alternative protein sources for fish farming, thus overall reducing the environmental impact. Within this scenario, the newRIFF project aims (i) to rear black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor*) on by-product-based diets consisting of by-products of paddy rice processing and other locally-available, organic by-products; (ii) to evaluate the impact of different diets containing increasing levels of insect meals as replacement of conventional protein sources (i.e. soybean meal and fish meal) in rainbow trout farming, by evaluating fish productive performance, nutrient digestibility, overall health status, and gut health; (iii) to investigate the consumer acceptance and economic, environmental and social performance of rainbow trout farming by replacing conventional protein sources with insect meals that are produced using rice by-products and other organic by-products as insect rearing substrate; and (iv) to identify the best practices regarding the use of insect meal as alternative protein source in fish feeding and to summarize all the information gathered during the project in order to develop guidelines and policy recommendations. The newRIFF project is supported by Fondazione CARIPLO by the call “Circular Economy – Promoting research for a sustainable future – 2022”.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Aquaculture waste conversion by black soldier fly larvae: quality and safety of the reared insects

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Aquaculture waste (AW) may represent an important treat to the environment whether not properly disposed. A recent study suggested that fresh aquaculture waste could satisfactorily be treated by black soldier fly larvae (BSFL). However, daily collection of such waste might not be suitable in industrial facility. The present study aimed to evaluate the ability of BSFL on converting industrial AW. Five substrates consisting in different percentages (0, 25, 50, 75, 100%) of AW (dry matter = 21.43%) and chicken feed (dry matter = 90.11 %) were prepared and offered to BSFL (feeding ratio = 0.46 g-DM/larvae). Growth and bioconversion performances, as well as chemical quality and microbial safety of the larvae were evaluated. Increasing the percentages of AW in the initial substrate led to an increasing of ash and indigestible fibre, while percentage of lipids decreased. Decreasing of insect growth performances were observed when amount of AW in diet was increased (from 126.28 mg on 0%AW to 72.60 mg on 75% AW) and absence of growth was detected when 100% AW was used. Larvae raised on substrates containing high amounts of AW showed lower lipids (from 35.52-0%AW to 17.27-75%AW) and proteins (from 40.62-0%AW to 35.87-75%AW), while the amount of ash increased (from 11.03-0%AW to 31.74-75%AW). Fatty acids profile of BSFL appeared to be stable and characterised by a predominance of lauric acid, while amino acids of BSFL mirrored the rearing substrate. High microbial contamination (>8 log CFU/g) was always detected in the larvae, although no pathogens were found, with the exception of *Listeria monocytogenes* in insects growth in substrate containing 50% of AW. Such results suggested that although AW can be satisfactory converted by BSFL, a quality-improvement component (such as chicken feed) is required. High-quality insects could be obtained, which might be suitable as animal feed.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Bioaccumulation of β-carotene in black soldier fly larvae: a novel approach to functional nutrient upcycling

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The short reproductive cycle of insects enables high-yield biomass production in a short period and hence emerges as a promising protein and lipids source for animal feed ingredients. The black soldier fly larvae (*Hermentia illucens*) (BSFL) are among the most sustainable sources of animal feed ingredients. In our study, we investigated the BSFL bioaccumulation of β-carotene, a provitamin A carotenoid essential for poultry feed. Two different sources of β-carotene were used in this study. The first source was orange peel (OP) with 1.97 ppm initial β-carotene content. The second was a commercially available β-carotene powder (BC) with 3,930 ppm initial β-carotene content. BSFL were fed oil palm waste biomass based diets supplemented with 10% OP or 1% BC and also a control diet with the oil palm waste only which initially contained 10.77 ppm β-carotene content. Samples were collected in triplicates and the β-carotene contents were quantified in feed, dried larvae, and frass utilising the Ultra Performance Convergence Chromatography-Mass Spectrometry (UPC2 TQ MS) method. The matrix-matched calibration approach was used to consider the background interference contribution of the matrix to the quantitative analysis. The results showed that 1% BC supplementation had the highest larval β-carotene content of 224.90 ppm (SD = 16.26). Besides that, control BSFL fed with an oil palm biomass feed exhibited measurable β-carotene bioaccumulation of 24.40 ppm (SD = 16.26). The 10% OP supplementation did not result in larval β-carotene bioaccumulation. In conclusion, BSFL can bioaccumulate β-carotene from commercial concentrates and oil palm waste, an approach with potential applications in adding value to insect ingredients in animal feed.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Suitability of different agro food by-products for the rearing of *Tenebrio molitor*

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The use of insects as novel foods is a response to the need for sustainable protein sources alternative to the traditional ones. The growth substrate used to mass rear insects plays an important role in reducing rearing costs and environmental impact, influencing growth performance and their chemical composition. The use of agro-industrial by-products for insect growth allows their valorisation and is fully in line with the circular economy policy promoted by the European Union. In this work, funded by the EU project "ON Foods – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods" (NRRP, N° 1550), five by-products were evaluated for the growth of *Tenebrio molitor*: potato waste, okara, brewer's grains, hemp waste and tomato peel. Wheat bran was used as a control. Two types of tests were carried out: one using the substrates as they were and the other using 50% of by-product and 50% wheat bran (mixture). Survival, final weight per larva, weight gain, development time, yielded biomass, substrate conversion indexes and chemical analyses were assessed. The yield of larval biomass was significantly higher with the mixed substrate ($P < 0.001$). However, the interaction between the type of substrate and the factor (mixed or as-is) was also significant ($P < 0.01$). The lowest larval biomass (potato waste: 2.06 ± 0.07 g; okara 3.00 ± 0.28 g) was obtained in replicates with higher mortality, while longer development times in potato and hemp waste (potato 90 ± 4 d; hemp 54 ± 4 d). Overall, the mixture led to similar results to those obtained in the control, in contrast to what was observed when using as such, in particular potato and hemp waste. The brewer's grains gave good results for growth performance and mortality, while okara showed high mortality (55.2%), despite it allowed a fast growth and high final larval weight. Only by skilfully mixing the different by-products will it be possible to obtain best results for the production of larval biomass and enable the valorisation of waste.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Life-history traits of black soldier fly larvae fed dairy manure with probiotic treatments

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The world population is expected to reach 9 billion people in the next few decades leading to an increased need for agriculture production including livestock which is expected to double. With the increase in dairy cattle production, also comes an increase in waste production leading to higher financial strain on dairies for waste disposal and increased risk for bacterial contamination. This highlights a need for better waste management strategies. Black soldier fly larvae (BSFL), *Hermetia illucens* L., feed on a variety of decomposing organic matter, and are mass reared using these substrates to produce an alternative protein source that can then be fed back to livestock. Manure is one such substrate that can be a feedstock for BSFL; however is not currently approved in some parts of the world for use a feed for BSFL due to safety concerns. Previous research used *Arthrobacter* AK19, *Rhodococcus rhodochrous* 21198 at both the benchtop and industrial scale in Gainesville diet and found a significant effect in larval weight and bioconversion rate across the treatments. We aimed to adapt this work to use in dairy manure. This research aims to examine the life history traits of BSFL fed on dairy manure with different probiotic treatments to see if they improve the digestion and bioconversion. Larvae were reared on fresh dairy manure with 3 different probiotic treatments: *Arthrobacter*, *Rhodococcus*, and a combination. Larval weight was measured daily until weight either remained constant or started to decrease for 3 days. At that time the bioconversion rate and average larval weight were recorded and examined for statistical differences. Despite previous work showing a significant effect of probiotic treatment when added to Gainesville diets, we saw no significant difference between the dairy manure control and the treatments. This leads to the need for further optimization of the protocol for future use at the industrial scale due to the challenges associated with working with dairy manure. The addition of the probiotic treatments could allow BSFL to more efficiently digest dairy manure and convert it to a high protein source.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Turning restaurant leftover food into edible insect feed

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Leftover food has been used for livestock feed, and there is limited information on the use of leftover food for edible insects' feed. The specific objective of this project is to study the viability of leftover food for edible insect rearing. One-week-old crickets (*Gryllodes sigillatus*) and their diet (wheat bran) were purchased from Fluker farms and reared in a growth chamber at 166 crickets per $15 \times 12 \times 25$ cm^3 container at 28 ± 1 °C, 60% relative humidity. Leftover food was collected from restaurants as vegetable- and chicken-based foods and kept in a refrigerator until use. Following the 1980 Swine Health Protection Act, and USDA-APHIS, VS (1990) mandates plate leftover food shall be heated throughout at boiling (212 °F (100 °C) at sea level) for 30 minutes before the leftover food used for animal feed to eliminate any other harmful pathogens. The leftover food collected from restaurants was autoclaved to comply with this act and mandate. The autoclaved hot, wet, viscous food was cooled, blended, and dried. They were pelletized in granules to simulate the purchased wheat bran and used for the experiment. The crickets were fed regular food, such as wheat bran (control), vegetable-based, and meat-based pellets for seven weeks. Randomly selected ten crickets were weighted once a week, and the protein content was measured in the seventh week. The feeding trial was replicated at least four times. There was no statistically significant difference in weight, growth rate, and protein content among the crickets fed with wheat bran and leftover vegetable and vegetable chicken mix diet. The Cricket nymphs fed with wheat bran (control) had a higher weight (1.4 g) than the crickets fed with a leftover vegetable (0.8 g) and vegetable chicken mix diet (1.2 g). Similarly, higher growth rates and protein content were recorded in the crickets fed with wheat bran than those fed a leftover vegetable and a vegetable chicken mix diet. The crickets seem to receive the nutrients they need for growth and development from the leftover food, so we didn't see a significant difference in body mass gain, growth rate, and protein content when compared with the crickets fed with the regular wheat bran. This research could benefit insect-rearing companies looking for alternatives to traditional feeds.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Cellulose-degrading bacteria improve efficiency in the digestion of dairy and chicken manure by black soldier fly larvae

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Black soldier fly larvae (BSFL) have potential utility in converting livestock manure into larval biomass. However, BSFL have limited ability to convert dairy manure (DM) rich in lignocellulose. Our previous research demonstrated that feeding BSFL with mixtures of 40% dairy manure and 60% chicken manure (DM40) provides a novel strategy for significantly improving their efficiency in converting DM. However, the mechanisms are unclear. In this study, we conducted a holistic study on the taxonomic structure and potential functions of microbiota in the larval gut and manure during the DM and DM40 conversion by BSFL, as well as the effects of BSFL on cellulosic biodegradation and biomass production. Results showed that BSFL can consume cellulose and other nutrients more effectively and harvest more biomass in a shorter conversion cycle in the DM40 system. The larval gut in the DM40 system yielded a higher microbiota complexity. Bacillus and Amphibacillus in the BSFL gut were strongly correlated with the larval cellulose degradation capacity. Furthermore, in vitro screening results for cellulolytic microbes from the larval guts showed that the DM40 system isolated more cellulolytic microbes. A key bacterial strain (LB110; *Bacillus subtilis*) with high cellulase activity from the larval gut of DM40 was validated for potential industrial applications. Therefore, mixing an appropriate proportion of chicken manure into DM increased the abundance of intestinal bacteria (Bacillus and Amphibacillus) producing cellulase and improved the digestion ability of BSFL to cellulose-rich manure through changes in microbial communities composition in intestine. This study reveals the microecological mechanisms underlying the high-efficiency conversion of cellulose-rich manure by BSFL and provide potential applications for the large-scale cellulose-rich wastes conversion by intestinal microbes combined with BSFL.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Novel disposal of lignin-rich olive pomace residual to produce high-nutrient insect biomass

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At the global level, the European Union is the first producer, exporter, and consumer of olive oil. Large amounts of olive pomace residue (OPR) produced from olive oil manufacturing are considered as unwanted industrial effluents and pose serious environmental problems globally, especially in the Mediterranean region. Black soldier fly (BSF) larvae are used to reduce and reutilise organic wastes to decrease animal feeding costs. In this study, BSF larvae developed and converted this neglected cheap source to nutrient-rich biomass in their bodies. The rearing of BSF pre-pupae on 75% OPR substrate led to an increase in the conversion efficiency of lauric acid (79.76%), and palmitoleic acid (65.05%, Omega-7), also BSF reduced approximately 19% of OPR and converted it to 22% biomass. Therefore, the current study is considered as a novel sustainable solution for the disposal of OPR to produce integrated feed additives using BSF larvae.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Effect of vinegar waste on the main growth performances of black soldier fly larvae

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Black soldier fly larvae (BSFL) are bred worldwide for their garbage and organic waste management capabilities. The vinegar industry appears to be an important economic market since the production of by-products and waste is well developed in this industry. The aim of the research was to evaluate the inclusion of vinegar by-products (V) in BSFL diet and assess their effect on the larvae's growth performance. This by-product represents the residue of the clarification phase and normally is a biomass difficult to dispose. It is called filtration earth and it is used with the aim of eliminating suspended particles and optimizing the clarity of the vinegar. This research represents a starting point on evaluating filtration earth as component of BSFL diet, so after preliminary trial, the inclusions of 2.5% (2.5V) and 5% (5V) were assessed to determine a vinegar prudential inclusion threshold. The experimental design foresaw two negative control diets, a commercial one based on BEF recipe (BEF) and a chicken one (CD); and two experimental diets using BEF diet as main component and organizing four replicates per treatment. Interestingly, the main results showed no impact of V on BSFL mortality between all treatments ($P > 0.05$), while differences were recorded between CD, 2.5V and 5V (122 ± 6.10, 92.5 ± 6.03 and 86.3 ± 8.56 mg) for final individual weight. The total biomass did not show differences among V treatments and the other diets. The reduction of the wet residue showed high differences between the BEF (42.2 ± 1.21%) and CD (51 ± 2.32%) and 5V (49.3 ± 1.44%). Instead, the dry residue did not show the effect of V on the reduction operated by BSFL. The data highlighted that is possible to include the V in BSFL diet up to 5% without affecting the growth performances, while the reduction on wet basis is well represented by the inclusion of V. Future research is needed to assess the effect of V on the chemical composition of BSFL and to evaluate higher inclusion of V in the diet of BSFL, up to 20%.

INSECTS FOR WASTE MANAGEMENT AND BIOACCUMULATION

Enrichment of black solder fly (*Hermetia illucens*) larvae with omega-3 fatty acids using fish offal and seaweed

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Black solder fly larvae (*Hermetia illucens*; BSFL) have received a growing attention as a sustainable ingredient in feed production. However, BSFL is a very poor source of omega-3 fatty acids, which limits its usefulness as a feed source. This study evaluated the potential to enrich BSFL with omega-3 fatty acids by feeding four omega-3 rich ingredients: yellowfin tuna (*Thunnus albacares*) offal and three seaweeds (*Kappaphycus alvarezii*, *Gracilaria salicornia* and *Sargassum wightii*). Eight substrates were prepared using poultry manure as basal ingredient: a substrate containing 100% poultry manure (control); four substrates supplemented with 12% fish offal, *K. alvarezii*, *G. salicornia* or *S. wightii*; and three substrates supplemented with 6% fish offal and 6% *K. alvarezii*, *G. salicornia* or *S. wightii*. A total of 5-days old 3,600 BSFL were randomly distributed into 24 plastic containers (150 larvae per container) and fed with one of the eight substrates for 14 days (n = 3). At the end of the experiment, weights of BSFL were recorded. Fatty acid compositions of substrates and BSFL were measured. Omega-3 fatty acid (C20:5 EPA and C22:6 DHA) contents were significantly ($P < 0.05$) higher in the BSFL fed 12% fish offal. *K. alvarezii* and *G. Salicornia* also showed similar results, but only when fed together with fish offal. Further, the results showed a significant ($P < 0.05$) positive correlation between omega-3 contents in the substrate and BSFL. *S. wightii* did not enrich BSFL with omega-3 fatty acids. The BSFL fed 12% fish offal had significantly ($P < 0.05$) higher growth performance and bioconversion efficiency than the control group. The supplementation of fish offal in combination with *K. alvarezii* or *G. Salicornia* caused similar growth performance and bioconversion efficiency in BSFL as in control group. In conclusion, supplementation of poultry manure with fish offal and seaweeds (*K. alverazii* and *G. salicornia*) can enrich BSFL with omega-3 fatty acids without compromising larval performance.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Results of testing a software powered training program for BSFL production in three countries

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For BSFL to meet global demand price-competitively is crucial, especially in developing countries. Despite efforts by some insect companies to scale BSFL production through semi-automated factories, challenges remain in competing with traditional feeds like soy and fish meal, due to high operational costs. In many cases a smaller scale farm that does not need of sophisticated infrastructure is the only option to utilize BSFL. Many aspiring insect farmers and companies face challenges across all production stages. Access to support, training, and resources is critical for their success as most have little or no experience with BSFL or background in biology. These farmers and teams require a structured, cost-effective support system that enables them to scale their operations profitably, with realistic and risk-minimizing milestones for business model validation and fundraising. This study reports results of a training program supported by a software solution developed by Manna Insect. The solution was tested in three countries: Finland, India and Kenya with different scale operations. In each case employees with little BSFL production experience were trained with a systematic program and the production results were noted during each training week. The program included a full production cycle from breeding to harvest larvae and rearing them to pupa and back to flies. After the initial training they continued operations using the developed software. The evaluation of the program success was done based on three key metrics: number of eggs collected per cage surface area, egg to 5dol larvae survivability in nursing and how systematic FCR was from 5 dol to 12 dol. The guidance on how to measure and log each metric was part of the training program with guidance of each task and tools needed. The results showed that it is possible to train anyone for professional level BSFL production, if they can be supported after the initial training and they learn to log the critical metrics correctly. Additionally it was concluded that controlled climate conditions help significantly on production stability and efficiency compared to outdoor production. The test farms were estimated profitable even at the level of one ton of live larvae per month.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Methodology in BSF conversion: evaluating the influence of *Homo sapiens* on a step-by-step bioconversion protocol

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The interest in converting low quality organic waste with black soldier fly (*Hermetia illucens*, BSF) larvae has increased as the demand for insects as protein source has risen. Yet, due to the relative novelty, in some countries, BSF conversion of post-consumer food waste still faces regulatory challenges. As new research areas, including micro- and molecular biology, start to deepen the knowledge within BSF conversion, the technology is inching towards the final goal of creating a circular economy, in which post-consumer food waste can be used by large scale industry. Now more than ever, there is a need for concise methodologies that support BSF research carried out in a controlled, reproducible and comparable manner. General guidelines to standardize BSF conversion and industry-scaled protocols are available, but waste-specific protocols that offer a step-by-step description of the general methods on lab scale are lacking. Also, to the author's knowledge, the robustness and reproducibility of such a waste-specific protocol has not been assessed before. Differences in treatment performance are often attributed to factors, such as T, larval density, feed substrate or genetics. Yet there is one more element for possible differences in results: the human factor. Therefore, a step-by-step, lab-scale protocol, specifically for the treatment of Northern European restaurant waste (NERW) was established. The protocol was conducted by participants with varying hours of experience in BSF conversion (prior BSF work experience ranging from 0 h-15.000+ h). The participants were provided the same NERW (25 ± 1% DM), seed larvae (1.8 ± 0.3 mg), treatment area (26 ± 1 °C and 40 % RH), tools and protocol to guide them through a simple feeding experiment in triplicates. The study highlights a difference in results between individuals with varying experience, such as estimation of larvae number (at start and harvest), sampling, feeding and harvesting. Variations were found across participants but could not always be contributed to the level of experience. The results of this study can be used to further establish and improve methodologies across BSF labs, both in academia and industry.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Biofeasibility as a pivotal step to empower the industry for waste-to-protein conversion

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In Europe, an estimated 20% of the total food production, equivalent to 88 million tonnes annually, is currently lost, incurring an associated cost of approximately 143 billion euros. Notably, about 30% of this food waste occurs during processing and manufacturing stages, generating substantial organic byproducts, ranging from 5,000 to 10,000 tons annually for a typical food manufacturer. EU countries are committed to the United Nations sustainable development goal of reducing food waste by 50% by 2030. Many companies have prioritised the reduction of food waste as a core sustainability objective. The integration of insect farming has emerged as a prospective solution to mitigate food waste and promote a circular economy. This abstract delves into the potential of utilising black soldier fly larvae (*Hermetia illucens*) for waste to protein conversion. Through a comprehensive evaluation of bio feasibility with the latest available data, the abstract demonstrates the economic and sustainable viability of BSF larvae factories. Bio feasibility is a critical process in maximising the utilisation of BSF larvae for waste upcycling. The process begins with a detailed analysis of the physical properties of the available material. Moving to feed formulation, creating diverse types of feed, rigorously testing and optimising for the best bioconversion potential. Scaling up to industrial mass, the chosen feed undergoes more testing, refining the processes for large-scale efficiency. In the quality check stage, final products like protein powder, lipids, and frass are thoroughly assessed through certified laboratory analyses. Factory planning is then informed by the resulting mass balance. Upon completion of bio feasibility testing, tailored recommendations are provided to optimize the utilization of black soldier fly larvae and inform sustainable factory planning for waste upcycling. Reference: https://www.eufic.org/en/food-safety/article/food-waste-in-europe-statistics-and-facts-about-the-problem FUSIONS 2016 https://mycorena.com/mycotalks/circular-concepts-for-tackling-industrial-food-processing-waste, https://sdgs.un.org/goals/goal12

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Larvae dosing methods: spreading vs spot application of seed larvae in rearing containers influences BSFL performance

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The bioconversion efficiency of black soldier fly larvae (BSFL) on organic waste streams to larval biomass is affected by various factors such as the physical properties of the waste, nutrients of the waste material, and the larval rearing process. We posit that when seed larvae (also known as 5-day-olds) are dosed into final rearing trays, the methods of dosage may influence performance outcomes. Early instar larvae are highly sensitive to minor changes in their feeding environment, which can impact survival and growth rates. This study investigates the effects of two dosing methods spreading and spot application within rearing containers on BSFL performance. The spreading method was achieved by spreading homogeneously larvae on the entire surface of feed in the container used. The spot application was achieved by accumulating the larvae in the middle of the feeding container using a dosing scoop, the area not exceeding 4 cm^2. Our results indicate that the spreading techniques resulted in significantly ($P < 0.05$) smaller larvae size (0.05 g vs 0.07 g), although with a higher survival rate (80% vs 54%) compared to the spot method. The rearing period was also reduced by two days with the spreading method. By increasing the area of larvae spreading on the feed, the survival rate was improved although it led to a low individual larvae size, likely less feed availability per larvae. Further research is needed to understand the cause of mortality in the spot method and to explore the potential role that BSFL density-dependent feeding behaviour could play in these results.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Collaboration in insect farming through open data models: a shared resource for farmers and researchers

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Interest in farming insects for food and feed is increasing exponentially. Insect farms are now being designed with IoT and AI technology integrated from the start, so a standardized approach to data modelling has become essential. We are developing data models tailored to insect farming, designed to contribute to an open data commons, along with example templates for compatible data ownership and licensing agreements. The objective is to streamline data collection, enhance compatibility, and foster collaboration within the insect farming community. This session will cover the creation of data models for critical aspects of insect farming, including environmental conditions, taxonomy and classification for ML systems, life cycle, habitat, nutrition, health, farm management, production metrics, environmental impact, and economic factors. This system serves as a flexible framework for organizing and representing insect farming data. The collaborative development process involves input from entomologists, agriculturists, and data scientists to ensure the practicality and relevance of the system. This initiative aims to establish a shared resource, enabling farmers and researchers globally to access, contribute, and derive insights from a collective repository of knowledge in insect farming. To promote community engagement and expedite progress, we will release these data models as open source. With ownership and license agreements, we also consider that data is valuable, and that farmers now need to protect their intellectual property similarly to software engineers and data scientists.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Innovative organic waste and agriculture residue preparation in decentralized insect farming facilities

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The growing demand for sustainable protein sources has led to increased interest in decentralized insect farming as a viable solution for organic waste management and protein production. This study introduces a novel and innovative method designed for the efficient processing of organic waste in decentralized insect farming facilities, focusing on size reduction, mixing, and conditioning of feedstock. The proposed method employs a combination of advanced shredding and blending technologies, optimizing the particle size of organic waste for enhanced insect digestion and nutrient absorption. By employing precision in the size reduction process, the method ensures a homogeneous mixture of diverse organic materials, fostering a nutrient-rich substrate for insect growth. The study investigated, on an industrial scale, an innovative approach to feed processing for decentralized insect farming units. A feasibility study was conducted, examining parameters such as handling, personnel requirements, feed quality, and economic viability. Preliminary results indicate economic potential for this new approach in decentralized insect farming facilities. The approach enhances flexibility in the acceptance, storage, and processing of various organic wastes, whether liquid, semi-solid, or solid, while reducing investment costs. In particular, with regard to poorly storable substrates, an improvement in handling and mixing was observed. In conclusion, the presented methodology offers a novel approach to the management of organic waste in decentralized insect farming facilities. The integration of advanced size reduction, mixing, and conditioning techniques may not only enhances the feedstock preparation but also demonstrates potential economic and environmental benefits for sustainable insect farming practices. Further research and implementation of this method hold the potential to increase flexibility and reduce investment costs.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Metrics in industrial insect farming: selection, definition, measurement and variability

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The last decade has seen a surge of both academic and commercial activity on insect farming and in particular the farming of the black soldier fly (BSF, *Hermetia illucens*) as a sustainable protein ingredient at an industrial scale. To realise its impact potential, insect protein has to be able to compete on price and value with more unsustainable legacy protein sources that can build on decades of value chain optimisation and economically often also benefit from unpriced externalities (social and environmental) or legacy subsidies (e.g. fertilizers, diesel inputs, tax breaks, etc.). Therefore, for insect farming to be successful and competitive, production factors need to be consistently and efficiently managed and optimised. You can't improve what you do not measure – developing a framework of input and output metrics with consistent definition, measurement, reporting and integrated interpretation is an important foundation for data-driven operations. Our review of the published literature reveals a wide variety of metric selections, definitions, measurement and reporting-basis that make comparability and repeatability often difficult and sometimes impossible. There also is a gap in (credible) public performance data from commercial insect production operations that would allow collective benchmarking. At the same time variability and the lack of repeatability is a recognised challenge among researchers and industry. A selection of examples in research design, metric selection, measurement and reporting that we encountered in published literature is presented (from an industry player perspective). Consequently, we propose a framework of metrics to facilitate repeatability and comparability between experimental data focusing on the bioconversion process (rearing of larvae from seedling to target stage) as a key focus area of optimisation. Finally, we offer a reflection on how a data-driven operations framework can be integrated and operationalised in commercial facilities using automation and software-aided solutions.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Impact of prepupae population density and container size on BSF prepupae weight loss and pupation

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The black soldier fly (BSF) undergoes holometabolous development, where successful pupation requires both feeding and weight gain during larval stages as well as adequate pupation conditions. In adverse pupation conditions, BSF prepupae may display extended pupation site selection behaviour, resulting in individual weight reduction and unsuccessful pupation. This study aims to assess how pupation container size impacts weight loss and pupation rates of BSF prepupae. A cohort of 3,072 prepupae were distributed among three sizes of round containers without pupation substrate, a design found to promote constant movement. Each container housed 128 prepupae, while a control group experienced optimal pupation conditions in small containers with pupation substrate. After 10 days, individual pupal weights and total pupation rates were recorded. Weight reduction in prepupae significantly increased with container size. The control group, with a population density of 2 prepupae/cm^2 and pupation substrate, exhibited the least weight reduction (14.72%) and the highest pupation rate (94.77%). Prepupae at the same density without pupation substrate experienced slightly higher weight loss (16.37%) with a comparable pupation rate (94.39%). Containers with 0.4 prepupae/cm^2 demonstrated significant weight loss, recording the highest reduction (19.79%) and the lowest pupation rate (86.33%). The adult emergence and sex ratio were also measured although no significant differences were found across all treatments. These findings suggest that the absence of pupation substrate and low prepupal density extended pupation site selection behaviour, leading to continuous movement and subsequent weight loss in prepupae. Consequently, the presence of pupation substrate and appropriate container size emerge as critical factors in minimizing weight loss and maximizing successful pupation.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

From the idea to an operative protein plant: planning approach and best practice from the project management perspective

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Insect farming and cultivation, particularly of species such as black soldier flies and mealworms, has gained significant attention as a sustainable solution for various industries, ranging from animal feed production to waste management. However, the complex nature of insect farming presents unique challenges in terms of planning and execution. This presentation delves into the intricacies of planning insect farming projects, highlighting the need for intense multidisciplinary collaboration and effective project management techniques. Insect farming projects encompass diverse sizes and constraints, necessitating tailored planning strategies. The planning horizon for insect farming ventures is extended, further complicated by the dynamic nature of assumptions and constraints. This fluidity underscores the requirement for a hybrid approach, combining traditional waterfall methodologies with agile project management techniques. The utilization of both methods ensures adaptability while maintaining a structured framework. However, effective planning is only one facet of project success. Stakeholder management emerges as a critical factor in achieving favourable outcomes. Engaging stakeholders, addressing concerns, and aligning expectations are paramount to fostering cooperation and support throughout the project lifecycle. This presentation not only offers insights into the intricate planning processes involved in insect farming projects but also presents a compendium of best practices. By examining common pitfalls and lessons learned from practical experiences, this paper equips project managers with valuable knowledge to navigate the complexities of insect farming initiatives. In conclusion, a comprehensive and adaptable planning approach, rooted in multidisciplinary collaboration, is pivotal for successful insect farming projects. By embracing a hybrid project management methodology and prioritizing stakeholder engagement, project managers can forge a path towards successful execution, ultimately ensuring the contentment of all stakeholders involved. This presentation synthesizes the core tenets of insect farming project management, providing a roadmap for prosperous ventures in this burgeoning field.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Development of a mealworm proximate composition analysis system using a handheld spectrometer in the NIR region

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Recently, there has been a surge in research focused on feed insects. In feed formulations, nutritional analysis is conducted for individual ingredients such as corn and soybean. Recent technological advancements allow precise measurement of distinct nutritional components for each crop within the feed, facilitating meticulous blending ratio adjustments. To effectively incorporate insects in practical feed applications, further research is needed on various non-destructive detection technologies and scale-up studies in a factory setting. Spectrometer-based non-destructive sensing technology, in this context, holds significant advantages for discerning the nutritional components of feed insects. Mealworms (*Tenebrio molitor*), among other species, are gaining prominence as a key feed insect and are being recognized as a suitable variety for future industrialization. This study introduces the development of a portable spectrometer tailored for quality measurement and monitoring of dried mealworm products. Farm-specific samples were collected, placed in 100 mm Petri dishes, and analyzed using spectral information in the 1000-1,700 nm NIR range obtained from the portable spectrometer system. Proximate composition analysis, covering moisture, crude protein, crude fat, and crude fiber (%), provided reference values. Utilizing these references, a partial least square regression (PLSR) model was developed for prediction. PLSR determination coefficients (R2) for moisture, crude protein, crude fat, and crude fiber exceeded 0.8, with root mean square error values indicating results within 5%. These promising outcomes suggest the feasibility of future in-line installation systems, paving the way for a robust feed quality monitoring system tailored for individual insects.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Effect of the testing scales in BSF research

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The scaling up of the BSF industry is underway, with many industrial actors announcing facilities producing more than 1,000t of protein per year. Such yields can only be achieved with huge facilities, e.g. Innovafeed's factory is planned to produce 15,000- t of protein per year for a footprint of 25,000 m^2. This means many millions of individual insects at any given time. While the industrial rearing and mating conditions differ between actors, the insects live in large units: Typically, both mating cages and rearing trays/conveyors are filled with anything between 10^4 to 10^6 insects. On the other side of the spectrum, R&D testing is conducted at a much smaller scale for obvious cost reasons. Most of the literature relates rearing tests with hundreds to a few thousand larvae, and mating tests involving from a dozen adult flies to a few hundreds. Due to these differences, it is no surprise that some results obtained at small scales are not replicated at larger scales. Therefore, there is a genuine risk of defiance within the community about test results obtained at smaller scales which may or may not be replicated at larger scales. Reasons for these differences remain to be fully understood, but the obvious candidates for larvae rearing are heat generation and homogeneity of the growing medium, while for mating, flying space and number of potential mates likely have a significant impact. This communication illustrates some differences observed between the rearing and mating scales observed at Innovafeed and provides recommendations on how to derisk tests conducted at small scale. More specifically, comparison of feed conversion ratio and total biomass produced in trays of 2 kg and 10 kg are compared with those produced in batches of more than 300 kg. These results are obtained with dedicated semi-industrial testing capacities. The results show slightly lower performance at higher scale and larger variability.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Using Artificial Intelligence to measure crickets, monitor colonies and scale experimental design

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Precision livestock farming (PFL) technologies are efficient, non-invasive monitoring systems that aim to optimize livestock rearing by tracking individual performance variables using state-of-the-art artificial intelligence. Population growth and increased food demand have rendered PLF a vital tool in ensuring sufficient food for our rapidly growing population. Using DeepLabCut, we trained a deep learning algorithm to automatically measure the head width, thorax width, and thorax length of crickets in both individual and group settings. Additionally, using Bug Mars' Hexapod technology, we were able to collect data 24/7 on crickets raised at different densities. This technology enabled us to elucidate granular insights about cricket growth and physiology, demonstrate the viability of using artificial intelligence tools to streamline insect research, and conduct experiments that would otherwise be too laborious, time-intensive, or complex to run manually. Finally, we present a cost-benefit analysis, discussing the merits of automating data collection. Our findings provide a path towards integrating industry-scale insect precision livestock farming technologies into commercial rearing practices.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Validation of a low-cost system for comprehensive characterization of black soldier fly bioconversion

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Treatment of heterogenous urban biowaste with black soldier fly larvae (BSFL) can produce sustainable protein- and fat-rich larvae as valuable animal feed. Efficient and reliable bioconversion with biowastes and low emissions (e.g. CO_2, NH_3) are desirable for highest economic and environmental outcomes. Thus, science and industry need an accessible research platform to study the complex bioconversion processes comprehensively under conditions resembling industrial-scale settings. Therefore, a novel low-cost rearing reactor for BSFL bioconversion was designed, tested and replicated (n = 4) to conduct feeding experiments under controlled, industry-scale mimicking conditions. It consists of a fan-ventilated chamber (630 mm × 850 mm × 620 mm) with three industrial rearing crates and air in- and out- ducts for optimal sensor deployment. Metabolic processes during the bioconversion are sensed automatically (every 2 min): substrate temperature (3 sensors per crate), larval plus substrate weight, NH_3 in the outgoing air as well as CO_2, air temperature, and relative humidity in the in- and outgoing air. The design was validated by tests on CO_2 recovery, air tightness, airflow homogeneity and BSFL performance with chicken feed and food waste. Low across reactor variability for all performance parameters underlined its reproducibility and outstanding suitability for substrate comparison studies (substrate: food waste, dry matter based, stdev: standard deviations, cv: coefficients of variation): BCR 1 % stdev, individual larval weight 1 mg stdev, total larval yield 2 % cv, total frass yield 3 % cv, total CO_2 production 7.7% cv). The novel setup is a unique opportunity for science and industry to thoroughly assess the bioconversion process. Thereby it contributes to a better understanding of the characteristics of BSFL rearing under different substrate and rearing settings (e.g. larval density, feeding rate) which will lead to an improvement of its reliability and sustainability impact.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Lessons learned from using paused life-cycle BSF neonates in various industrial setups

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As the black soldier fly industry enters the scale-up and maturation phase, there is a growing need to increase production efficiency, quality and consistency, while reaching desired market capacity and price parity with other protein sources such as fish meal. While first-generation companies took a vertically integrated approach, encompassing breeding, rearing, waste management, and processing, newer market entrants and existing players have recognized the operational and financial advantages of transitioning to a specialized, decoupled model. This model involves centralized BSF breeding (reproduction) hubs that supply multiple rearing facilities. PauseM® represents a major innovation milestone to achieve simpler protein production in this evolving landscape. It offers pre-counted, ready-to-use packages of paused life-cycle neonates, featuring proprietary technology that ensures a minimal shelf life of 14 days in a user-friendly application. Over the past few years, we have distributed PauseM® units to a variety of protein production sites worldwide, ranging from small-scale R&D farms to full-scale production facilities. These pilots resulted in valuable insights on protein production efficiency and standardization. These include insights about the flexibility of BSF in consuming a wide variety of feedstuff, the ability to significantly shorten the rearing phase and increase yield at industrial scale, whilst reducing operational costs. I will present some of the pilots and key findings, as well as discuss future implications of the PauseM® technology on the future of effective insect farming.

INSECT PRODUCTION SYSTEMS ENGINEERING, DIGITALISATION AND AUTOMATION

Deep learning-based segmentation for silkworm growth monitoring in insect farmings

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The escalating global demand for insect-based food, driven by population growth and year-round food requirements, underscores the need for efficient insect farming practices. Despite their environmental benefits, insect farming encounters challenges in monitoring growth, mortality, and pest damages, relying on time-consuming and labour-intensive methods. This study proposes a deep learning-based segmentation algorithm as a foundational step towards developing a comprehensive monitoring system, with a specific focus on silkworm growth stages. Silkworms play a pivotal role in the evolving sericulture industry, transitioning from silk production to a source for edible products, necessitating a monitoring system to ensure product quality during this shift. The segmentation algorithm addresses diverse feeding and harvesting requirements based on silkworm sizes and growth stages. Yolov8 deep-learning models were employed in the experiment, utilizing three silkworm types ('Baek-gyeon,' 'Hwang-gyeon,' 'Sammyeon-zam'). Data sourced from 'AI-hub' in Korea formed the dataset. Results indicate a segmentation accuracy exceeding 0.97 for each class at mAP50, highlighting the system's efficacy in classifying growth stages and ensuring high-quality product production. This research establishes a foundational algorithm for a monitoring system, poised to guide crucial actions such as feeding and harvesting based on growth stages and pest infection. Future studies aim to enhance the monitoring system's capabilities, with the ultimate goal of practical implementation in the insect farming industry. The robust results attained in this experiment mark a significant advancement towards the real-world application of the proposed monitoring system.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Comparison of ultrafiltration/diafiltration and isoelectric precipitation to produce a yellow mealworm protein isolate

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The low consumer acceptance for edible insects in Western countries is the biggest challenge for the insect industry. Nevertheless, the use of insect protein-rich ingredients, such as protein isolate could enhance acceptability. Insect protein isolate could be produced by isoelectric precipitation (IP) and ultrafiltration/diafiltration (UF-DF) but the efficiency and impact on the insect matrix of these two processes were never compared. Consequently, this work aims to compare the impact of UF-DF and IP on the composition, structure, and techno-functional properties of the mealworm protein isolate. Our results showed that a lower protein content was obtained for UF-DF (72.20%) than IP (82.08%), whereas similar protein extraction yields were calculated. No difference in the protein profile of both isolates was noticed. A lower surface hydrophobicity (106.78 vs 493.50) and a higher denaturation temperature (181.44 °C vs 161.32 °C) were obtained for IP compared to UF-DF. Moreover, solubility was higher for UF-DF from pH 2 to 8 whereas similar values were calculated from pH 9 for both UF-DF and PI isolates. No difference was observed for foaming properties (pH 7 and 9), with low foaming capacity and no foaming stability for both isolates. Emulsion stability measured at pH 7 and 9 was higher for UF-DF isolate with respective values of 28 and 34 min compared to 6 and 15 min for IP. Emulsion droplet size was not affected by the pH and was higher for IP compared to UF-DF (2.65 vs 0.86 µm). We demonstrated that the processing had a major impact on the techno-functional properties of mealworm protein isolate. Moreover, higher protein content was obtained in the IP isolates compared to UF-DF. However, due to protein denaturation induced by precipitation, the IP isolates was characterized by its lower techno-functionality. The next step will consist of to compare the potential of both isolates as ingredients for the development of model food systems.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Gut microbiome of *Zophobas morio*: isolation, antimicrobial activity and radiosensitivity to e-beam

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Limited research has been conducted on the radiation processing of edible insects to enhance both food safety and functionality. Thus, we investigated the application of electron beam technology to mitigate microbiological hazards in the gut microbiome of *Zophobas morio* (superworm), an edible insect. A total of thirty-one isolates were identified, including representatives from Enterobacteriaceae, Pseudomonadaceae, and Lactobacillaceae families, confirmed by 16 s rRNA gene sequencing. Notably, *Lactobacillus garviaea* and *Weissella paramesenteroides* exhibited significant antimicrobial efficacy against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The radiosensitivity analysis under a 2.5 MeV-electron beam demonstrated D10 values ranging from 0.08 to 0.10 kGy for Enterococcaceae and Pseudomonadaceae. Complete inhibition of microbial activity was achieved at an absorbed dose of 0.8 kGy, highlighting the potential of food irradiation to enhance food safety and extend the shelf life of edible insects. This study emphasizes the efficacy of food irradiation in reducing the population of foodborne pathogens, positioning edible insects as a valuable avenue for future research and development in the domain of sustainable food sources.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Impact of different insect processing technologies on microbiological product quality

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Prior to use as food, feed or other applications, insects typically undergo processing to improve their microbiological safety, stability and technological properties. Several processing methods can be employed, each influencing end product quality. Differently processed insects may harbour a different microbial load, impacting food safety. This study evaluates the impact of five processing technologies for mealworms and/or black soldier fly larvae (BSFL) on microbiological product quality: hybrid microwave (μW) and radio frequency (RF) drying, low energy electron beam (LEEB), high moisture extrusion (HME) and meal production after tricanter centrifugation (TCM). Microbiological aspects related to the processing technologies were assessed through general and pathogen-specific analyses. Findings revealed that all processing methods could significantly reduce certain groups of vegetative microorganisms (e.g. total viable counts < 7 log cfu/g, Enterobacteriaceae < detection limit), thus improving overall microbiological quality of the end products. However, effectiveness against bacterial endospores, typically more resistant to processing, varied for each technology applied, with LEEB and HME being the most effective. Notably, the pathogen *B. cereus* was detected in a few BSFL meals. Although all investigated processing technologies improved the microbiological quality of the insects, achieving the desired microbiological quality and safety may require extra decontamination steps. Also, it is crucial to monitor for specific pathogens like *B. cereus* during and after insect processing.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Insect meal fractionation using sieving technology

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Insect meal is usually obtained directly from the insect larvae through different process operations comprising in a variable order a defatting step, a drying step and a milling step. The quality of this insect meal comprises two important criteria: its protein content and the protein digestibility, but also other criteria such as ash content, fat content, amino acid (AA) profile…In the perspective of better adapting the insect meal quality criteria to the consumer needs, we therefore describe a sieving process that allows an easy modulation of the composition of the resulting insect meal fractions. In this process, separation of the different meal fraction is conveniently positioned after defatting and drying of the coarsely (<8 mm) crushed whole larvae, and can therefore be easily adapted to existing wet rendering processes. After sieving defatted and dried BSF (black soldier fly) meal through a 1 mm mesh, two fractions of equivalent weight are obtained. The low size fraction has a higher protein content than the large size fraction (+15%, sum of AA, on DM), a higher ash content (+75%, on DM), a lower chitin content (–75%, ADF-ADL method, on DM), and a better protein digestibility (+12%, Boisen). Moreover, the low size fraction might better fits the nutritional needs of salmonids, as its AA profile is richer in essential AA compared to the other fraction. Insect meal is mainly constituted of the larvae inner part (pulp) and of the outer part (cuticle). The latter is rich in chitin, a polysaccharide polymer that gives the cuticle its rigidity. Cuticle proteins are mainly embedded into this rigid matrix. By sieving coarsely crushed processed larvae, we therefore separate cuticle pieces (larger in size) from the rest of the larvae components. Recent research mentions promising effects of the presence of chitin on the gut health of various species (shrimps, salmonids, pets…). Thus, there is an interest for the differentiation and recombination of different protein meal fractions to adapt insect meal formulation to the specific needs and benefits of each species, which is easily achievable with our sieving process.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Optimizing *Tenebrio molitor* larvae meal for sustainable meat analog production

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The global shift from traditional animal protein consumption to alternative sources, including plant-based and unconventional proteins like algae, insects, and pulses, is a critical response to the challenge of feeding a growing world population. This study focuses on a pivotal aspect of this transition by investigating the partial substitution of plant proteins with *Tenebrio molitor* larvae meal. Our objective is to contribute to resourcing diversification and establish a correlation between the physicochemical properties of *Tenebrio molitor*, and their suitability for high-moisture extrusion cooking. This is a key step in formulating environmentally friendly ingredients with meat-like organoleptic properties. The study involves the defatting of insects using supercritical CO_2 and subsequent high-moisture extrusion-cooking experiments with varying incorporation rates of insect flour in a formulation based on pea protein isolate and wheat gluten. Our aim is to determine the optimal insect incorporation without compromising the anisotropic and fibrous structure necessary for a meat analog. We measured the impact of lipids, chitin and powder rheology on the extrusion process to establish recommendations and standards for functional insect meal dedicated to meat analog production. Texture analysis, including tensile tests and dynamic mechanical analysis, along with macroscopic and microscopic examinations of extrudates, provides insights into the final product's characteristics. Results indicate that defatting process by supercritical CO_2 improve flowability index by 23 points and floodability by 8 points. Notably, our study reveals a strong correlation between the fibration capacity of Tenebrio Molitor meal and its lipid content. This finding advances scientific understanding in the domain of insect proteins with other components such as lipids and their texturing capabilities.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Quality and safety evaluation of *Tenebrio molitor* paste submitted to high hydrostatic pressure processing

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In order to promote the implementation of edible insects within the modern food system, high safety standards should be guaranteed. High Pressure Processing (HPP) is an emerging non-thermal technology capable of reducing microbial contamination of food while preserving its nutritional quality. The present study investigates the impact of HPP on quality and safety of *Tenebrio molitor* paste. Three different pressure levels (200, 400, 600 MPa) and six times were selected (0 min–control, 5, 15, 30, 45, 60 min) and applied on paste generated after milling fresh larvae. Microbial investigation of the studied pastes revealed a significant reduction in total viable count (TVC) at pressures of 600 MPa and 400 MPa (initial count = 8.49 cfu/g), with final counts ranged between 6.42 cfu/g (5 min) and 4.11 cfu/g (60 min) for the treatment at 600 MPa and between 7.61 cfu/g (5 min) and 6.26 cfu/g (60 min) when a pressure of 400 MPa was applied. No microbial reduction was observed at 200 MPa. Slight although significant increase of titratable acidity was detected at every pressure level, with the higher acidity measured for paste treated at 400 MPa for 60 min (7.57 ± 0.34 mL NaOH/g sample). Complete absence of lipid oxidation was detected at the studied pressure levels, with the peroxide value going through a slight, although significant reduction regardless by the treatment time. Similar behaviour was also observed for TBARs at 600 MPa. Increasing of TBARs from short to long treatment time was instead observed at 400 and 200 MPa. Total phenol content and antioxidant activity, evaluated through DPPH and FRAP methods, increased notably after the HHP treatment, with the highest values recorded at 400 MPa, regardless by the treatment time. Reduction of lipase activity was also revealed at every pressure level, with an evident linear effect following the treatment time. Treatment at 600 MPa for 15 min was identified as the best combination for ensuring significant microbial reduction while keeping high nutritional quality of mealworm paste.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Improving protein solubility of *Alphitobius diaperinus* with food grade nutritional supplements

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Wet fractionation is a commonly used method in producing food ingredients from edible insects. The main output of this method is the soluble fraction, which consists mostly of soluble proteins, for products such as protein powders. By increasing the protein yield within this fraction, it is possible to reduce the number of insects required for production. Free amino acids enhance protein solubility, especially in biochemical and pharmaceutical uses. This study explored the utilization of free amino acids L-arginine and L-lysine in the fractionation process of both blanched and non-blanched *Alphitobius diaperinus* larvae. The tested amino acids were introduced to the insect matrix during the wet grinding phase prior to centrifugal fractionation. In the case of non-blanched insects, the soluble protein yield was enhanced by all treatments involving various concentrations (0.5-1 M) of the amino acids, ranging between 21-43% increase. Among these treatments, 1 M L-arginine achieved the highest protein yield. These treated samples exhibited a significantly greater volume of the soluble fraction during wet fractionation. However, for blanched insects no consistent protein solubility increase has been found. The soluble fractions treated with amino acids have demonstrated a protein distribution by molecular weight that is comparable to the control fractions, with the addition of some unidentified proteins. The treated samples demonstrated a significant increase in protein surface hydrophobicity, which, in conjunction with the enhanced protein solubility, suggests less protein aggregation. Moreover, preliminary tests suggest that the inclusion of L-arginine and L-lysine in the blanched soluble fraction leads to a significant improvement in microfiltration protein permeability. These findings exhibit that the use of amino acids not only enhances soluble protein yield but also modifies the physiochemical properties of insect-based proteins. This novel combination administers a highly soluble and fortified insect food ingredient that maximizes the utilization of the edible insect for a variety of applications. Future research should assess techno-functional properties and cost-effective alternatives.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Pulsed electric field (PEF) processing of house crickets: microbiology and protein properties

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House crickets (*Acheta domesticus*) are already consumed as food. Pulsed electric fields (PEF) represent a non-thermal food processing technology capable of disrupting cell membranes in foods, thereby improving microbial stability, enhancing conventional processes, and altering protein structure and functionality. The study investigated the effect of PEF pretreatment (4.4 kV/cm, 500 pulses) on the production of cricket flour. Different drying methods, namely hot air drying (60 °C, 4.5-5.5 hours) and freeze-drying (48-96 hours), were applied to assess their impact on the quality characteristics of the resulting flour. PEF pretreatment reduced the total energy consumption of hot air drying by 18.04% and freeze-drying by 49.82%. Microbiological evaluation and assessment of protein properties were conducted on the produced flour. PEF conditions alone were insufficient to achieve satisfactory microbial inactivation in fresh crickets (0.4 log.g-1, 0.4 log.g-1, 0.2 log.g-1 for mesophilic bacteria, Enterobacteria, and molds, respectively). However, when combined with hot air drying, a significant reduction of 2.7 log.g-1, 1.6 log.g-1, and 2 log.g-1, respectively, was observed, indicating a substantial effect of the drying conditions. No reduction in spores was noted under any condition. Regarding protein properties, PEF exhibited varying effects depending on the drying method. PEF treatment did not induce protein oxidation, which was influenced by the drying method (37.62 and 19.83 nmol/g protein for hot air and freeze-dried flours, respectively). When applied to freeze-dried crickets, PEF increased the disulfide content of proteins from 12.20 to 16.37 µg cysteine equivalent/mg protein and surface hydrophobicity from 73.52 to 105.68. Similarly, the colloidal properties (turbidity, zeta potential) of proteins were mainly influenced by the drying method. Despite the synergistic influence of PEF and drying method on the product quality and safety, several challenges need to be addressed, like elevated production costs and the low consumer trust on the technology and the use of insects as food.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Microbial dynamics in extruded cricket-enriched porridge flour: a study on storage-induced changes

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Extrusion cooking is a common processing method for cereal grains, and understanding its impact on the microbial profile of the resulting flour is crucial for ensuring food safety and quality. This study delves into the microbial dynamics of extruded cricket-enriched flour (CEF) throughout four months of storage. CEF consisting of dehulled maize (30%), roasted millet (40%), cricket powder (24%), sunflower seed oil (5.8%) and micronutrient, was formulated to meet nutritional needs of infants and young children. Corn-soy blend (CSB), a standard fortified porridge for humanitarian programs, was used as comparison. The porridge flours were processed by extrusion after which the extrudates were ground into flour and packaged into one kilogram plastic containers, and stored at room temperatures. The microbiological load, pH and moisture content were monitored monthly for four months. Analysis of both flour revealed average moisture levels below the maximum limit of 12%, with a slightly acidic pH (5.51-6.18) in CEF, and no significant variations observed during storage. Total aerobic, total coliforms, *Staphylococcus aureus*, yeasts and moulds that exceeded the allowable limits were observed in non-extruded flours. However, a statistically significant reduction in all types of counts was observed after extrusion process. No changes were observed during the entire storage period except a significant increase in fungal counts in CEF at the end of storage period. The predominant fungal genera were *Aspergillus*, followed by *Fusariu* and *Penicillium*. Both flours were devoid of Salmonellae and *Escherichia coli*. The findings provide valuable insights into the microbiological stability of extruded porridge flour, contributing to the development of effective storage practices and quality control measures in the food industry.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

High-pressure processing for decontaminating soluble insect proteins preserves the protein nativity more than blanching

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Ensuring microbial safety and inactivating endogenous enzymes are required to produce high-quality insect products. Thermal processing like blanching is currently used in industry but can negatively affect techno-functional protein properties, necessitating milder alternative decontamination methods such as high-pressure processing (HPP). This study compares the effects of HPP at 200/400/600 MPa (10 min) to conventional blanching at 90 °C (10 min), on microbial inactivation and techno-functional protein properties. Soluble protein fractions (pH 3) from house crickets (HC) and lesser mealworms (LM) were used. The results showed that HPP significantly reduced the microbial load for both insect species, especially at ≥400 MPa with at least 2 log reduction for aerobic microorganisms and no detection of anaerobic microorganisms, yeasts, and moulds. However, blanching was more efficient in inactivating aerobic microorganisms. The protein molecular weight distribution and secondary protein structure were preserved better with HPP than with blanching, which explained why HPP was less effective in protease inactivation than blanching, although this was only evident in LM fractions. The emulsification and foaming properties were unaffected by HPP while blanching decreased the emulsification properties. HPP improved protein solubility after pH adjustment to 7 compared to untreated and blanched fractions. HPP, despite higher capital and operational expenditures, can be justified for products requiring high protein solubility and emulsification properties. Blanching, more economically favourable, is beneficial for products requiring proteolytic stability. To conclude, HPP and blanching effectively inactivate microorganisms in both LM and HC. The desired final product application should guide the choice when designing insect protein processing.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

High-pressure processing and fermentation for valorization of processing residues from edible insects

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Insects are highly nutritious food and feed source. Combination of blanching and mechanical fractionation techniques leads to production of high-value protein-rich food ingredients along with insect processing residues which are rich in chitin and chitin-bound proteins. This study proposes two approaches for valorization of insect processing residues and demonstrates their applicability by the example of residues from lesser mealworm (*Alphitobius diaperinus*). The first approach comprises high-pressure homogenization at 60 MPa and 120 MPa. The results revealed that the application of high-pressure changes in the microstructure and particle size of chitin-rich processing residues treated at 1% w/w. Pressure treatment also significantly improved emulsion stability (emulsion instability index of 0.54-0.68 vs 0.77 for untreated samples) and improved foam stability (78.08-79.67% vs 11.37% for untreated samples). Improvement in functionality was more pronounced at higher pressure treatment (120 MPa). The second approach uses lactic acid fermentation in order to remove proteins bound to chitin. It was determined that four lactic acid strains (*Limosilactobacillus fermentum*, *Levilactobacillus brevis*, *Lactiplantibacillus plantarum*, *Lacticaseibacillus paracasei*) can be used for fermentation insect residues, and 2 log growth of microorganisms was achieved as a result of fermentation at 37 °C. The results demonstrated partial deproteinization of insect residues already after 48 hours of fermentation with the mentioned strains, but a more pronounced effect was observed after 120 hours (up to 28%). Also, fermentation resulted in the solubilization of proteins and the formation of peptides with antioxidant activity. Thus, the tested strategies propose two distinguished ways for the sustainable valorization of insect processing residues for food and feed applications and can contribute to the overall sustainability of the insect production chain.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Persistence of *Salmonella infantis* in *Acheta domesticus* and *Tenebrio molitor*

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The interest toward edible insects' potential as an alternative protein source has increased in recent years. *Acheta domesticus* (AD) and *Tenebrio molitor* (TM) are considered among the insect species most suitable to be farmed for food purposes. Salmonella is recognized as one of the most important foodborne pathogens and, among the wide number of *Salmonella serovars*, *S. infantis* has recently emerged for its ability to persist especially along the poultry production chain. We reported in a previous systematic review (SR) that Salmonella spp. can persist in incomplete metamorphosis insects for more than 10 months and in complete metamorphosis for about 29 days. To date, few data are available on Salmonella risk in insect farming and to fill this gaps a study was carried out in order to evaluate the potential for *S. infantis* to colonize insects and persist during farming. The experiments were carried out on insects fed with two different diets (A and B) and at three developmental stages (age 1, 2 and 3). The results showed considerable variability in the acquisition of Salmonella by insects and in persistence. In the case of AD, the persistence of *S. infantis* observed varied between 2 days (age 2 diet A) and 15 days (age 2 diet B). In the case of TM, the persistence varied between 1 days (age 1 diet B) and 22 days (age 2 and 3, diet B). Such variability in the experimental results suggests that the non-homogeneous distribution of the microorganism in the feed and the reduced consumption of the feed (and thus microorganism) by insects may lead to an uneven spread of Salmonella in the different individuals. High persistence also suggests that if contaminated feed is consumed, there is a possibility for Salmonella to infect insects and be present at the end of the life cycle, although in general the two species studied are able to eliminate the infection over time. It is therefore important to consider the timing of introduction of Salmonella along life cycle. Scaling up production and differentiation of substrates will influence the risk of presence and persistence of Salmonella and other human pathogens in insect farms.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Assessing the development, nutritional profile, and techno-functional properties of BSF as a sustainable protein source

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Black soldier fly (BSF) larvae are being recognised for their capacity as a sustainable protein source and their rapid conversion of organic waste into biomass. Comprehending the technological and functional characteristics of BSF larvae at different phases of their life cycle is crucial for their effective use in diverse applications. This study assessed the life history parameters, proximate analysis, and techno-functional qualities of BSF larvae. The techno-functional properties included protein solubility, oil holding capacity, foam capacity and stability, emulsifying capabilities, and gelling properties. The BSF larvae decreased the amount of dry matter in the substrate by 49.26% over a period of 12 days. The survival rate of the BSF larvae was 94%, and the Feed Conversion Ratio was 1.63. The proximate study showed that protein, fat, and carbohydrate concentration varied among different life stages, with larvae having the highest fat content. Moreover, the BSF (larvae, pupa and adult) protein techno functional properties were analysed and it was noted that the adult shown increased solubility throughout the pH range of 9-10, indicating its potential suitability for diverse food and beverage items. Subsequent investigations indicated that the oil holding capacity of BSF larval protein was lower to that of pupae, which, in turn, was lower to that of adults. The foam volume was greatest in pupae, whereas larvae and adults had lower values. Furthermore, the protein derived from BSF larvae exhibited exceptional emulsifying potential. The research investigated the gelling properties using a heat-induced method, which uncovered specific temperatures at which gelation begins for larvae, pupae, and adults. Gaining a comprehensive understanding of these characteristics at different phases of life is essential for maximising their effectiveness in a range of applications, including as food and feed production.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Rearing substrate – the impact on safety of black soldier fly larvae and frass

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Currently in the UK and the EU the material that can be used as a rearing substrate for insects for production of protein for feed and food is regulated. Insects reared for this purpose are defined as farmed animals and may only be fed materials that are used for other farmed animals. Therefore, waste streams that contain or may potentially contain animal by-products (ABPs) (with a few exceptions) are not permitted to rear the insects. Regulators and policymakers require comprehensive risk assessments when considering the safety of feed and food. The potential chemical and microbiological hazards that may be present in larvae and frass will depend on the rearing substrate used and the environment in which the insects are reared. In this study, we have assessed chemical and microbiological contaminants of *Hermetia illucens* (black soldier fly) larvae reared on a selection of currently non-permitted rearing substrates, and in the remaining frass. The substrates tested were supermarket surplus containing ABPs, food processing waste containing ABPs, kitchen waste from a catering establishment and poultry manure. BSF larvae and frass produced using currently permitted substrates were supplied by a commercial BSF producer for comparative purposes. Analytical methods screened for chemical analytes (metals, veterinary medicines, pesticides, mycotoxins, PAHS, nitrate/nitrite, PFAS), the presence of key microbial organisms was assessed, and non-targeted screens were used to assess the presence of natural toxins and viral RNA present in the rearing substrates, the larvae and the frass. Key results and findings are discussed, and areas identified for further research are highlighted. The study has provided a key data set for subsequent risk assessments.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Production of insect protein hydrolysate with microbial fermentation

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The rearing of insects, especially of the black soldier fly (BSF), *Hermetia illucens*, is a promising strategy for converting otherwise lost nutrients back into protein and fertilizer, and to supplement local food security. The BSF has a high efficiency, a lower footprint and less greenhouse gas emissions compared to conventional livestock and a protein content that is higher or comparable to conventional food groups. Despite the evident advantages from both sustainable and nutritional standpoints, negative perceptions associated with the appearance are a barrier to its widespread adoption as food. One effective way to overcome consumer acceptance challenges is to develop refined insect-based protein ingredients that can be readily included in familiar foods. This would require extensive understanding of the functional and nutritional properties of the insect constituents for formulation and development. Our research tackles this opportunity by extracting and purifying the protein constituent of the BSF larvae and tailor the nutritional property for human diet by altering the amino acid composition and bioavailability through microbial fermentation. The larvae material was pasteurised and pre-processed prior to inoculation with bacterial strains for fermentation. The process was optimised for desirable amino acid profile and ash and fat content. The optimised fermentation process increased the amino acids content, especially the essential amino acids. The impact of the protein hydrolysates/fermented end products on human health was evaluated using an ex-vivo model system whereby biomarkers produced by the gut microbiota that are linked to health were examined using metabolomics. It was concluded that this process holds promise for producing high-quality, safe, functional and nutritious BSF larvae-derived food ingredients that can be readily included in formulated food products elevating their health and nutrition benefits, while also overcoming consumer acceptance challenges.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Protein quality of black soldier fly larvae (*Hermetia illucens*)

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Black soldier fly larvae (*Hermetia illucens*; BSFL) production allows for utilization of by-products from food and feed industry to produce protein rich meals being used in feeds for farm animals, aqua species and pets. In this context protein quality, which is defined by the amino acid (AA) composition, is of importance. In order to characterize protein quality of BSFL meal, 83 samples were analysed for crude protein (CP) and amino acids (AA) by wet chemistry in our lab. Later on samples were split into 30-40 (C1; n = 8), 40-50 (C2; n = 13), 50-60 (C3; n = 45), 60-70% CP (% in dry matter; C4; n =17) categories. Accordingly, CP content differed significantly among groups (35.2, 46.9, 54.1, 65.0% CP; $P < 0.01$) as did Lys, Met+Cys, Thr, Arg, Val, Ile, Leu, His, Gly, Ser, and Glu ($P < 0.05$) and was less pronounced for Ala (no difference between C1 and C2) and Asp (difference between C2 and C3; $P < 0.1$). However, when regressed against CP, slopes varied largely between AA from 0.009% (Cys; $r^2 = 0.52$) and 0.091% (Glu; $r^2 = 0.63$). While relatively low r^2 values indicate quite some variation of data, r^2 for Lys, Leu, Val, Ser, and Pro regressions were 0.90 or higher. Interestingly, Phe content did not differ among groups C1, C2, and C3 but it was significantly higher in C4 ($P < 0.05$). However, while AA levels basically increased with CP, ratios to Lys (AA profile) behaved different. Ratios of Met+Cys, Thr, Val, Ile, Leu, Gly, and Ser to Lys were highest in C1 samples ($P < 0.05$) and similar among the other CP groups. Arg and His to Lys ratio was only marginally changed, while Phe and Pro to Lys ratio showed lower ratios in C2 and C3 than in C1 and C4 ($P < 0.05$). Overall, these analyses demonstrate that AA levels and protein quality, expressed in the AA profiles, varies with protein. A rather high variation within data set suggests that using averages or table values for precise feed ingredient evaluation needed for farm animal feed formulations would be suboptimal. Therefore, Evonik Animal Nutrition developed an NIR calibration (AMINONIR®) for rapid analysis which captures the entire AA profile as well as crude protein and other crude nutrients.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Exploring the flavourful world of edible insects and their aromatic building blocks

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Understanding the flavour of edible insects is crucial for consumer acceptance, guiding both product formulation and marketing strategies. The variability in volatile compounds, influenced by species, developmental stages, food processing, and analytical techniques, underscores the need to characterize and comprehend flavour profiles of edible insects. Taking ants as an example, our study demonstrated the diverse flavours of edible insects and their potential as unique flavouring ingredients. The analysis of volatile compounds in leaf cutting ants (*Atta mexicana*), common black ants (*Lasius niger*), spiny ant pupae and adults (*Polyrhachis* sp.), and weaver ant workers and queens (*Oecophylla smaragdina*) using headspace solid-phase microextraction and gas chromatography-olfactometry-mass spectrometry revealed distinct odour profiles for each ant species. Common black ants exhibited a pungent, acidic, and vinegary smell attributed to their high formic acid content in venom glands. Similarly, adult spiny ants contained formic acid, while the pupae did not, reflecting changes in venom gland development during metamorphosis. In contrast, chicatana ants presented nutty, roasty, woody, and fatty notes. Unlike Formicine ants, chicatana ants lacked formic acid but featured 4-methyl-3-heptanone as an alarm pheromone and 2,5-dimethylpyrazine as a trail pheromone. The fatty aroma of chicatana ants was linked to aldehydes such as hexanal, octanal, and nonanal, while the nutty aroma was attributed to pyrazines. Weaver ants were characterized by a nutty, sweet, and caramel-like aroma, contributed by various pyrazines and pyrroles. However, undesirable hay and urine-like off-flavours were also detected, likely due to high concentrations of amines. Understanding these intricate flavour profiles is imperative for the food industry to develop insect-based products that not only appeal to consumers but also overcome psychological barriers, such as disgust-based aversions, associated with insect consumption.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Microbial load dynamics of black soldier fly larvae reared on heterogeneous urban food waste in Singapore

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While valorisation of homogenous food side streams has gained traction around the world, the potential of heterogenous food waste, contributing to 11% of the total waste in Singapore, remains underexplored. In part, this is due to the variability and complexity of its composition, posing challenges in upcycling and raising food safety concerns for downstream applications. Employing voracious feeders like black soldier fly larvae (BSFL) presents a novel solution through its efficient waste elimination and ability to yield valuable ingredients in feed or food production. This study assessed the microbial load in BSFL (n = 3) fed surplus heterogenous food waste, pre-and post-processing. Three batches of food waste, collected from a residential dining hall at the National University of Singapore over different periods were incorporated in a single-feeding experiment. Larvae were then processed via microwave drying and defatted. Raw larvae revealed a high microbial load (total aerobic count) of 6.0 ± 0.5 Log CFU/g which was not significantly different from the heterogeneous waste (7.3 ± 1.0 Log CFU/g, $P = 0.130$). Industrial heat treatment of larvae significantly reduced microbial load by 1.9 Log (3.9 ± 1.1 Log CFU/g, $P = 0.045$). While Enterobacteriaceae was identified in raw larvae (2.7 ± 0.7 Log CFU/g) they were below the detection limit in both heterogeneous waste and heat-treated larvae (<100 CFU/g). In processed larvae, 9 other microbial parameters, including *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Bacillus cereus*, *Salmonella* spp., *Listeria monocytogenes*, *Cronobacter* spp., and *Campylobacter* spp., were within the permissible limit set by the microbiological standards in Singapore. Overall, findings offer valuable insights into surplus waste management by BSFL within residential halls, highlighting a significant reduction in microbial load in processed larvae regardless of the substrate's microbial load. This potentially transforms waste into microbiologically safe ingredients for downstream processing.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Physical characteristics of dehydrated house cricket (*A. domesticus*) powder and associated volatile flavour compounds

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Edible insects could provide a sustainable alternative protein source, but recent consumer studies suggest that flavour may be a limiting factor when fortifying foods with edible insect powder. This study investigated the impact of oven-drying, a key processing step in edible insect powder manufacturing, on house cricket powder properties, colour and flavour development. Dehydration ensures a microbially stable powder product (water activity, $a_w < 0.6$). However, heat driven dehydration may cause physical changes and chemical reactions leading to colour change and flavour development. Several physical characteristics were studied at hourly intervals (0-6 hours) to assess the impact of oven-drying conditions at various temperatures. Moisture content was analysed using thermogravimetric principles, and colour determined with a CIELAB colorimeter, a modified tristimulus analysis yielding values for dark/light (L*), red/green (a*), and blue/yellow (b*) to enable sample comparison. Solid-phase microextraction followed by gas chromatography-mass spectrometry was used to determine volatile compounds over time. Greatest water loss was observed during the first 2 hours for all temperatures, achieving moisture content of 1.33-15.02 g/100 g. The rate of water loss reduced after 3 hours and was only time dependent; temperature had no impact ($P > 0.05$). Changes in a_w followed a similar trend, and microbial stability ($a_w < 0.6$) was achieved at a faster rate at higher temperatures (within 2 hours at 90 °C and 100 °C). Significant changes in L* (darkening) were observed at all temperatures (greatest at 100 °C), indicating the occurrence of Maillard reactions, which was also supported by the presence of volatile compounds such as pyrazines, pyrroles, pyridines, furanones, furans and aldehydes. In conclusion, colour and flavour develops as dehydration proceeds, introducing attributes in oven-dried cricket powder that may carry through to food matrices during reformulation.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Effects of storage conditions and drying methods on the physicochemical properties of crickets and mealworms

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Edible insects present a promising and sustainable protein alternative, offering both environmental and nutritional advantages. To establish their reliability as food sources, it is essential to investigate shelf stability. In this study, we examined the physicochemical changes in mealworms and crickets over a 3 month storage period at different temperatures (25 °C, 4 °C, and −18 °C), employing various drying methods (microwave, oven-drying, freeze-drying) to determine the optimal approach. Crickets stored at 4 °C on day 14 exhibited lower TBARS (0.21 μg/g) than the other days (1.87 to 1.58 μg/g) and pH (6.86), accompanied by high crude protein content (52.53%). Similar trends in TBARS and pH values across all samples were observed until day 35. Mealworms also displayed significant changes in oxidative indicators (TBARS and pH) on day 35. Concerning storage temperature, microwave processing resulted in higher moisture content (mealworm: 6.61%, cricket: 4.09%), but lower crude protein content (mealworm: 48.40%, cricket: 66.89%), while oven-drying produced the highest crude protein values (mealworm: 52.27%, cricket: 70.15%) and exhibited higher antioxidant capacity (mealworm: 37.35%, cricket: 35.54%). Based on the results, the optimal storage conditions for both mealworms and crickets were identified between days 14 and 35, across all temperature conditions. Furthermore, oven-drying could be the best drying method for edible insects in terms of high protein content and antioxidant capacity.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Unveiling nutritional, health, and allergy potential of black soldier fly larvae protein using INFOGEST *in vitro* digestion

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Including insects in food and feed requires understanding their nutritional quality, digestibility, health benefits, and potential allergens. This study assessed these features for five types of black soldier fly larvae (BSFL) protein from three companies processed as a whole and defatted BSFL powder. We applied proximate analysis, total amino acid profiling, in vitro human gastrointestinal digestion, discovery proteomics, and bioinformatics to evaluate the nutritional quality, digestibility, peptide physicochemical properties, and to map allergens and bioactive peptides. Notably, BSFL protein content varied between 35% to 55%, and the defatting process enriched the protein content which contained ~40% essential amino acids. The total protein hydrolysis percentage was significantly different among the three companies (ranging from 23% to 39%), but no difference between whole and defatted BSFL powder. The simulated digestion not only significantly reduced the particle size and changed microstructure of BSFL samples but also altered the physicochemical properties of released peptides at molecular level. The number of identified proteins and peptides significantly increase during gastric stage, and then significantly dropped at small intestinal stage for all samples. Within each sample type, high similarities were noticed on the profile of identified peptides at the same digestive stage, whereas variations were observed between the whole and defatted samples as well as among the samples from different companies. The peptides detected during gastric and small intestinal digestion phases contain multiple intact bioactive such as anti-hypertensive peptides and allergen epitopes, including tropomyosin and arginine kinase epitopes in all samples.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Physicochemical, nutritional and structure properties of mealworm powders manufactured by different processes

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In Europe, edible insects are gaining interest due to their positive attributes on food security and environment. The main insect-based ingredients produced in the world are whole and defatted meals, which are obtained by a relatively simple process and are widely used in the food industry. The present study aimed to evaluate the effect of pre-treatment (blanching, freezing) and thermo-mechanical process (pressing + drying) of Tenebrio molitor larvae meal prepared at the pilot scale from: (1) fresh freeze-dried larvae meal (FFL), (2) whole (undefatted) blanched and dried larvae meal (WUL), and (3) partially defatted, blanched, pressed and dried larvae meal (PDL). The results showed a significant difference between meals belonging to the three batches based on their physico-chemical (protein, lipid, ash, chitin, carbohydrates, aw, and dry matter), colour (L*, a*, b*, C*, h, BI, and WI), technofunctional and molecular (fluorescence and mid-infrared) data sets. For example, the level of protein was 61.7, 52.9 and 51.4% for PDL, WUL, and FFL, respectively. The highest value of BI and L* was observed for FFL with 76.4 and 46.9, respectively. PDL samples showed the highest values of oil holding capacity (2.1 g oil/g meal), swelling capacity (2.7 mL water/g meal), and water holding capacity (2.7 g water/g meal). These differences observed at the macroscopic level were found to impact the secondary and tertiary structure of larvae meals. In conclusion, the above-mentioned findings underscore the importance of selecting processing technology for producing larvae meals according to their application in the food industry.

INSECT PROCESSING, PRODUCT CHARACTERISATION AND FOOD SAFETY

Structural and techno-functional properties of protein extracted from different *Tenebrio molitor* larvae flours

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Insects are being considered as a protein source, given their high nutritional value and sustainability. However, to gain acceptance in Western markets, insects need to undergo processing to be incorporated into food products. The functional characteristics of insect-derived ingredients are pivotal in choosing suitable products, taking into account factors like nutrition, taste, and safety. The extraction and processing of these proteins not only modify their composition but also their functional properties. This study investigates the physico-chemical characteristics, as well as the structural and functional properties of proteins extracted from *Tenebrio molitor* larvae flours originating from different materials: fresh freeze-dried larvae and two flours (whole and partially delipidated) obtained by different thermo-mechanical processes. Protein extraction by alkaline solubilization (45 °C, pH 10, ratio 1/10) yields 15% and 44% for flours and larvae, respectively. The protein contents of the extracted fractions are high (83.7% and 80.4%, respectively). Proteins extracted from flour have lower solubility properties compared to those extracted from larvae, with a minimum solubility index in the pH range of 3-5. Flours are more prone to aggregation and possess lower surface hydrophobicity than larvae. At pH 7 and 25 °C, the air/water and oil/water surface tensions are 31.5 and 5.2 mN/m for flour proteins, and 43.9 and 6.7 mN/m for larvae proteins. Proteins from flours and larvae exhibit similar foaming capacities, but foam stability of flours proteins is lower than that of larvae for concentrations <4%. The emulsifying properties of flours proteins are superior to those of larvae for concentrations <2%. Nevertheless, at concentrations of 4% for foams and 2% for emulsions, these proteins have foaming and emulsifying properties comparable to reference proteins such as bovine serum albumin or prolacta 95. These functional properties possessed by insect proteins thus offer the possibility of being used as foaming or emulsifying agents.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Consumer awareness and acceptance of insects as food and feed in Sri Lanka

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This study aimed to evaluate the consumer awareness and acceptance of insect-based food and feed in Sri Lanka, and to determine if the acceptance can be increased by provision of information. An online survey was conducted with 1,059 participants to determine consumer awareness and acceptance of insects. Among the participants, 93%, 43% and 33% were aware that the insects can be used as food, feed and pet food, respectively. Notably, males exhibited significantly lower awareness of insect-based food compared to females ($P < 0.05$). Of the participants, 47% expressed their willingness to consume food produced from animals fed with insects, while majority favoured consuming eggs from insects-fed chickens. On the other hand, 28% were willing to consume insect-based food directly. Male respondents and Buddhists displayed significantly greater acceptance of both insect-based food and feed than females and Hindus, respectively ($P < 0.05$). To study whether the acceptance of insect-based food and feed can be increased by the provision of information, a paper-based survey was conducted with 210 third year undergraduate students of the Faculty of Agriculture, University of Peradeniya, Sri Lanka. The students were randomly divided into three groups (n = 70): a control group (no information provided), a leaflet-informed group (information provided using a leaflet), and a video-informed group (information provided using a video). Results showed that there was a higher acceptance of insect-based food in the video-informed group compared to the control group ($P < 0.1$), while no significant difference was observed between the leaflet-informed and control groups ($P > 0.05$). Information provision did not significantly affect acceptance of insect-based feed and pet food ($P > 0.05$). In conclusion, there is a potential for embracing insects as a food and feed source in Sri Lanka. Further, consumer acceptance of insects as a food can be increased by video-based information provision.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

What predicts and prevents Singapore residents from participating in insect upcycling food waste projects?

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Sustainable food production is vital in urban settings like Singapore, where 90% of the food is imported. Singapore aims to achieve '30 by 30,' sustainably producing 30% of its local nutritional needs by 2030. However, this expansion would require increased imports of fertilizers and animal feed. Insect Upcycling Food Waste (IUFW) projects offer a solution by combining insect cultivation with sustainable food waste management. IUFW projects allow residents to contribute by donating separated household food waste, which nourishes black soldier fly larvae. These larvae convert food waste into valuable organic fertilizer (frass) for growing vegetables and high-protein fish food. Social acceptance is crucial for the success of the IUFW projects. To understand residents' motivations and barriers to participation, we conducted a national online survey using the Theory of Planned Behavior (TPB). We conducted a study to determine the salient beliefs of residents to join the IUFW projects. We expanded the basic TPB framework by incorporating additional variables, including social norms, nature-relatedness, health, and sustainable behaviours. We found three main salient beliefs. First are people's feelings about joining IUFW projects, including helping the environment, helping to reduce food waste in Singapore, and contributing to food sustainability. Secondly, we found that the government, environmentalists, and family members impact participants' decision to engage in IUFW projects. Lastly, we identified factors that facilitate or hinder participation, including convenient food waste collection points, the provision of free containers for segregating food waste, clear guidance, time commitment, incentives, hygiene concerns, and support from household members. Furthermore, our findings indicated that participants' attitudes exhibited the strongest correlation with their intention to participate, followed by subjective norm and perceived behavioural control. To enhance residents' involvement in IUFW projects, we recommend that the government concentrate on bolstering public attitude by highlighting the projects' contributions to environmental and food sustainability while simultaneously addressing the factors hindering participation.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Effect of geographical location, insect type and cooking method on the nutritional composition of edible insects

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Edible insects may be a sustainable source of protein and some other nutrients, especially for low economic status communities. The current study determined the influence of insect type, geographic location and cooking method on the nutritional composition of insects. The investigation would contribute to maximal derivation of the nutritional benefits of insects. Dried samples of four insect types, *Gonimbrasia belina* (mopani worm), Gynanisa caterpillar, termite soldiers/ workers, and termite alates, were procured from different street vendors across Vhembe district in Limpopo Province, South Africa. Insects bought in Vhembe markets were originally from Zimbabwe, Botswana, Zambia, and around Limpopo Province of South Africa. Dried *G. belina* samples were washed with cold water and subjected to the following cooking treatments: (1) raw (sun dried); (2) boiling in water for 30 minutes; (3) boiling in water with additional salt added for 30 minutes; and (4) fried with oil and salted. Each cooking treatment was performed three times and the mean of the replicate was taken. Generally, nutrient content varied significantly with insect type and geographic location ($P < 0.05$). Protein content varied from 40 g/100 g in termite alates to 69.75 g/100 g in termite soldiers/workers. Termite soldiers/workers had the highest iron content (range: 545- 629.5 mg/kg), whilst Gynanisa caterpillar had the highest zinc content (range: 122.14-150.33 mg/kg). Similarly, Gynanisa caterpillar had the highest levels of lysine (range: 0.80-4.53 g/100 g), threonine (range: 0.79-2.64 g/100 g) and isoleucine (range: 0.63-2.33 g/100 g). On the other hand termite soldiers/workers had the highest levels of valine (range: 2.20-3.47 g/100 g), leucine (range: 2.49-3.87 g/100 g) and phenylalanine (range: 1.38-3.55 g/100 g). Cooking method significantly affected nutrient retention. Boiling with salt added resulted in the highest retention of protein and total mineral content (ash), and, therefore, seems a suitable method for cooking insects. The findings indicate that, if optimally selected and cooked, edible insects can contribute significantly to the alleviation of protein, zinc, and iron deficiencies in target communities.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Developing cost-effective media for cellular agriculture via gut biome-assisted fermentation of black soldier fly

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The study aimed to simulate the natural protein breakdown processes in the fish digestive tract by subjecting black soldier fly larvae (BSFL) as a feedstock to microbial fermentation using gut microbes isolated from the blue catfish gut. In the first step, BSFL slurry was subjected to enzymatic digestion, using pepsin and trypsin to mimic animal digestive processes. The results showed a 2.8% degree of hydrolysis after digestion with pepsin and an additional 5.9% after digestion with trypsin. In the second step, the gut of blue catfish was dissected, homogenized, and added to the BSFL hydrolysates for anaerobic fermentation. Two fermentation approaches were tested, including the direct addition of gut homogenate to the hydrolysates (fermentation A) and the establishment of microbial cultures from the gut homogenate before fermentation (fermentation B). Both fermentations resulted in similar protein content and degree of hydrolysis. Furthermore, the microbial composition of the BSFL slurry before fermentation differed significantly from that of the blue catfish gut. Cultivation and fermentation led to a decrease in species richness, with the loss of important chitinase and protease-producing genera such as Pseudomonas and Clostridiaceae. However, there was an increase in Paraclostridium and members of the Enterobacteriaceae. In addition, the effect of fermented hydrolysates from BSFL on the proliferation of zebrafish embryo fibroblasts was tested in comparison to fetal bovine serum (FBS) in in vitro cell cultivation. Lower concentrations of FBS resulted in decreased cell density and altered cell morphology. The supplementation of hydrolysate B at high peptide concentrations had cytotoxic effects on the cells, while at lower peptide concentrations, it improved cell proliferation only in cultures with 2.5% FBS. However, the difference was not statistically significant. Similar results were obtained with hydrolysate A, and supplementation with BSFL hydrolysate controls before bacterial fermentation showed no noticeable improvement in proliferation or cell morphology.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Structural properties of black soldier fly larvae protein concentrates extracted via salting-in and salting-out

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The black soldier fly (BSF) larvae have been used for animal feed. Due to its interesting composition, BSF larvae have great potential to be further implemented in the human diet. Herein, this study aimed to investigate the effect of the salting in/out extraction techniques, coupled with ultrafiltration, on BSF larvae protein. The protein isolates were extracted using alkaline extraction and acid precipitation (AAF) assisted by salting-in (AAFSI), salting-out (AAFSO), and salting-in/out (AFFSISO) coupled with ultrafiltration. Zeta potential measurements suggested improved solubility in salting-out-treated samples. In the FTIR analysis of protein extracts obtained from AAFSO and AAFSISO, a clear N-H free stretching vibration was evident within the range of 3,500-3,200 cm^{-1}. The β-sheet and random coil structures were transformed into α-helix and β-turns by salting-out treatments, as demonstrated by circular dichroism, indicating a more compact and organized conformation. AAFSO and AAFSISO, had the highest content of ordered protein structures and exhibited the largest ΔH values, measured at 141 J/g and 137 J/g, respectively. The proteins obtained through salting-out exhibited higher thermal stability, as evidenced by TGA and DSC analyses. SDS-PAGE showed distinct protein distribution patterns, with salting-out treatments influencing molecular weight distribution. Moreover, the findings emphasise the significance of extraction methods in tailoring the properties of insect-derived proteins, further promoting their use in the food industry and beyond. Evaluating the functional properties of these proteins in diverse food matrices, coupled with sensory assessments, will be imperative for understanding their applicability and consumer acceptability.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Acceptability of black soldier fly larvae food waste bioconversion facilities (BFWs) in Singapore

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Black soldier fly larvae food waste bioconversion facilities (BFWs) hold immense potential for sustainable urban development. These facilities have the potential to significantly contribute to circular urban food systems by promoting resource recovery, minimizing landfill waste, adapting to local waste characteristics, and enhancing resource efficiency. While BFWs have demonstrated effectiveness in pre-consumer (agricultural) waste management, their acceptance in urban settings, particularly for residential food waste recycling, remains underexplored. This study delves into the factors influencing Singaporean residents' acceptance of BFWs, with a focus on public housing neighbourhoods. We engaged with 20 residents in semi-structured interviews, probing into their personal experiences, knowledge, associations, existing practices, and perceptions of risks and benefits concerning BFWs. Thematic analysis revealed that factors such as the aesthetic appeal of the facility, insect-related stigma, convenience, financial incentives, and connection to place significantly impact acceptance. We also assessed attitudes towards the proximity of BFWs to residences, uncovering a prevalent "Not in my backyard" (NIMBY) sentiment. Despite a general openness to BFWs in Singapore, residents exhibited resistance to facilities located near their homes. This discrepancy highlights the complex interplay of factors like proximity to waste drop-off points, time investment in waste segregation, sustainability concerns, and prior exposure to composting or urban farming, in shaping acceptability and NIMBY attitudes. Our findings suggest that for higher acceptance and reduced NIMBY sentiments, BFWs must be strategically placed in community gardens blending with the local context, and equipped to mitigate odour and pest risks, while offering financial incentives for initial engagement. This research provides a foundational understanding of the community's perceptions and expectations regarding BFWs in their local environment. The insights gained are instrumental in developing strategies to enhance acceptance and comprehension of this innovative approach to urban food waste management.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Nutritional, technofunctional and structural insights into black soldier fly larvae-derived protein concentrates

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Due to their protein content and balanced amino acid profile, edible insects have been described as an excellent alternative protein source to combat malnutrition. As the global population continues to grow, edible insects such as the black soldier fly larvae (BSFL) may contribute to food security. The effect of different protein extraction methods, i.e. alkaline solution and acid precipitation (BSFL-PC1) and extraction with an alkali (BSFL-PC2), on the nutritional, techno-functional, and structural properties of BSFL flours and protein concentrates were studied. The highest protein content (73.3%) was obtained under alkaline and acid precipitation extraction (BSFL-PC1). The sum of essential amino acids significantly increased ($P < 0.05$) from 24.9% to 38.2% due to the defatting process during extraction. Protein solubility was significantly higher in protein concentrates (85-97%) than in flours (30-35%) at pH 2. The emulsion capacity (EC) was significantly higher ($P < 0.05$) in the protein concentrates (BSFL-PC1 and BSFL-PC2) compared to the freeze-dried and defatted BSFL flours, while the emulsion stability (ES) was significantly ($P < 0.05$) higher in BSFL-PC1 (100%) compared with BSFL-PC2 (49.8%). No significant differences ($P > 0.05$) were observed in foaming stability (FS) between freeze-dried and defatted BSFL flours. Fourier transform infrared spectroscopy (FT-IR) analysis revealed distinct structural differences between BSFL flours and protein concentrates. This was supported by surface morphology through scanning electron microscopy (SEM) images, which showed that the protein extraction method influenced the structural properties of the protein concentrates. Therefore, based on the nutritional and techno-functional properties, BSFL flour fractions and protein concentrates show promise as novel functional ingredients for use in food applications.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Consumer acceptance of insects as fish feed in Singapore

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The burgeoning aquaculture industry, driven by the increasing demand for farmed fish, has witnessed a surge in fish feed and fish oil prices in recent years. In response, there has been a growing exploration of insect-based fish feed as a sustainable substitute for traditional fish feed. This environmentally friendly approach holds particular promise in urban settings like Singapore, where 90% of food is imported. However, the success of integrating insects into fish feed hinges on social acceptance. To gain insights into Singaporean consumers' preferences and willingness to pay regarding fish fed with insect-based diets, we conducted a Discrete Choice Experiment (DCE) using seabass as a case study. We also analyzed participants' perceptions of local fish and insect farming practices. Our findings revealed that Singaporean consumers displayed a lower willingness to pay (WTP) for fish raised on insect-based fish feed, including insects fed with plants, pre-consumer food waste, or post-consumer food waste, compared to traditional fish feed. Conversely, consumers were willing to pay a premium for fish fed with fish feed characterized by lower microplastic contamination and reduced CO_2 emissions during production. Locally produced fish feed was preferred over imported alternatives. We recommend that the government promote the environmental benefits and sustainability associated with such practices to boost consumer acceptance of products with insects as feed.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Modelling consumer choice in the purchase of insect-based, cultivated and plant-based meat products

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Livestock feed production alone occupies more than 40% of global cropland and contributes to over 10% of global emissions, prompting the research and development of more sustainable substitute protein sources such as plant-based, insect-based, and cell-cultivated meat. Despite rising interest in alternative meats and proteins, the challenge lies in low consumer acceptance and motivation to achieve commercially viable production levels. Efforts by countries such as Singapore to approve insect-based and cultivated meat sales would be futile with limited consumer acceptance of novel food sources. Given strong associations between food consumption and local customs, further exploration into the motivators and barriers to meat alternatives consumption, especially in geographical regions lacking similar studies is required. Consumers engage in more active deliberation when making purchase decisions for new and unfamiliar foods. To understand the trade-offs that consumers consider when buying meat alternatives with varying environmental impacts, we integrated a discrete choice experiment into an online survey involving Singapore's residents. Participants chose between nugget packets based on meat type, carbon footprint, saturated fat, biodiversity, food waste, and price. The influence of social norms on their choices were also investigated. Responses were analysed using multinomial logit models to identify preferences and willingness to pay for changes in attribute levels. Preliminary results suggest deeper aversions to insect-based products and respondents generally prioritise health benefits over long-term environmental wellness. In our presentation, we elaborate on how respondents weigh each attribute level against one another and the effects of a social norm message in alternative meat product purchases. We also offer insights into how different consumer groups perceive each meat alternative and compare insect-based products against other available alternatives. The findings can better inform public and private policies involved with the marketing and managing of alternative proteins supply chains.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Exploring the nutritional value of Thailand's traditional edible insect palm weevil (*Rhynchophorus ferrugineus*)

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The consumption of palm weevil (*Rhynchophorus ferrugineus*), is a common practice in various communities across Asia and holds significant nutritional promise. This study investigates the nutritional composition of the palm weevil, sourced from a commercial palm weevil farm in southern part of Thailand rearing sago palm weevil in plastic containers. Freeze-dried insect larvae from the final instar stage were analyzed (n = 30, composite sampling, and triplicate analyses) using standard methods outlined by AOAC. The findings revealed that the insect larvae exhibited a protein content of 32.7% and a high fat content of 55.8%, with mineral content comparable to, if not surpassing, that found in conventional animal-based foods. The weevil's protein content is characterized by a rich assortment of essential amino acids, 46.3% of the total amino acid content, making it a valuable source of quality protein. The high fat content, comprising more than 50% unsaturated fatty acids with oleic acid being the most abundant one, in palm weevil augments its caloric density, providing a sustainable and energy-rich dietary option. The essential minerals, such as calcium (81.9 mg), magnesium (246 mg), potassium (1,086 mg), phosphorus (328.2 mg), and zinc (6.4 mg), were present in notable concentrations per 100 g of dried matter, signifying their nutritional significance. High potassium and low sodium content of the insect could offer potential benefits for blood pressure regulation, cardiovascular health, and related physiological functions. Beyond its nutritional value, the sustainable production of palm weevil offers a dual benefit by not only addressing nutritional needs but also contributing to economic enhancement for local farmers.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Ranking 10 insect-based food products based on consumer acceptance, environmental and public health impacts

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Edible insects, as a new sustainable alternative protein source, have significantly promoted innovation in the food industry in recent years, though their food safety is also of great concern. Therefore, a multidisciplinary approach is highly desirable to develop safe, healthy and sustainable products that are acceptable to consumers. The objective of this study was to propose a quantitative multi-criteria decision analysis (MCDA) method to (i) assess four criteria (C) for each food product: consumer intention (C1), environmental impacts (C2), microbiological food safety risks (C3) and nutritional quality (C4), and (ii) to aggregate them and rank the food products accordingly. The method was developed and applied to 10 insect-based food products: biscuit, bread, pasta, chip, porridge, milk, burger patty, sausage and nugget. Each criterion was defined through a selection of sub-criteria (SC). C1 includes product preference (SC1.1) and daily consumption (SC1.2) with data obtained through the cross-cultural consumer survey and national dietary surveys. C2 was assessed by SC2.1 on CO2 emissions, SC2.2 on land use and SC2.3 on water use; with data sourced from the food environmental impact database and insect powders processing. C3 was estimated by considering the likelihood of contamination (SC3.1) and the severity of illness (SC3.2) of all potential microbial hazards. The calculation of C4 was based on a widely used algorithm for food nutritional classification in Europe: Nutri-Score (SC4.1 total energy, SC4.2 protein content, SC4.3 fiber content, SC4.4 saturated fatty acid etc.). After data collection and normalization, we assigned weights to the four criteria and SCs to perform a relative ranking of the 10 insect-based food products. This methodology provides a reusable tool to guide food-developers in designing safe, healthy, sustainable and acceptable insect substitutes. It also paves the way towards multi-criteria ranking for novel food products.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Cricket powder susceptibility to stored product moth infestation

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Edible insects for human consumption represent a new source of protein thanks to their nutritional properties and lower environmental footprint. The house cricket *Acheta domesticus* is one of the species authorised for human consumption, and falls into the category of novel foods. This species can be marketed as a powder used in the preparation of several foodstuffs such as biscuits, crackers, bread and protein bars. As other commodities, insect-based products could be susceptible to stored-product pest infestations. This research work is part of the European Union Project "ON Foods – Research and innovation network on food and nutrition Sustainability, Safety andSecurity – Working ON Foods" (NRRP, N° 1550). We assessed the capability of four species of stored product moths (*Plodia interpunctella*, *Corcyra cephalonica*, *Cadra cautella* and *Ephestia kuehniella*) to infest the cricket powder and complete their development. Moreover, we tested the fertility of the moth females emerged from the cricket powder. To this end, 100 eggs of each moth were used to infest a standard diet (SD) and a cricket powder (CP). For each species and diet, five replicates were set up. Each replicate was checked three times a week and adult emerged were counted, sexed and wing width measured. Finally, couples were formed to assess fertility. Only *E. kuehniella* did not grow on CP, while other species developed until the adult emergence, with different rate of survival and time to emergence. Adult wingspan was higher in the specimen emerged from SD. Considering the fertility of the females emerged from SD and CP, the mean number of new larvae was significantly higher for SD in *C. cephalonica* and *P. interpunctella*, while non difference was observed on *C. cautella*. These data showed that cricket powder can be attacked by the stored-product moths that are able to complete their lifecycle. As it already happens with other foodstuffs, it is crucial to pay attention to the infestation along the entire food chain, and in particular during the storage. Other studies will have to consider the susceptibility of the cricket powder when it is used as an ingredient in other food preparations or the possibility of infestation by other food pests such as beetles.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Exploring the effect of diet on the flavour of yellow mealworm (*Tenebrio molitor*) larvae

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Insect farming offers a promising solution for the valorisation of agri-food waste and by-products. While the bioconversion potential and effect of different feed substrates on nutritional composition have been well-researched, their impact on flavour is underexplored. *Tenebrio molitor* (TM) larvae (commonly known as yellow mealworms) are one of the most promising species for large-scale insect farming due to their favourable nutritional composition, low environmental footprint, and ability to digest a variety of low-cost feed substrates. This study investigated the effect of diets containing grape marc (a local agri-food by-product) on the nutritional composition (proximate composition, fatty acids, and amino acids), volatile compound profiles, flavour and consumer acceptance of TM larvae. Three diets were tested, including a wheat bran (WB) control diet and two grape marc (GM) enriched diets (GM and WB mixtures of 25:75 and 50:50). Based on changes in nutritional composition, larvae reared on the WB (control) and 50:50 GM enriched diets were selected for evaluation by fifty Australian insect-eating consumers. Hot-air dried samples in two formats (whole and a cracker with TM larvae powder replacing 10% wheat flour) were profiled using Rate-All-That-Apply and assessed for overall liking (9-point hedonic scale). Significant differences (P]} < 0.01) were observed in the flavour attributes of crackers, mostly described by changes in savoury characteristics and an increase in *bitterness* of the cracker containing TM larvae reared on the 50:50 GM enriched diet. No difference in overall liking was shown between the two diets, regardless of format. This presentation will share key findings and discuss potential implications in the context of a circular economy. Specifically, can we balance desirable sensory properties with the valorisation of local agri-food waste and by-products?

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

The effect of processing on the nutritional values of insects

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Although the nutritional value of edible insects has been studied extensively, adequate data regarding the effects exerted by processing methods on their chemical composition are scant. In this study, our objective was to assess the effects of boiling, roasting, oven drying, and microwave heating on the nutritional value of insects euthanised via blanching or freezing. Parameters of the culinary treatment were boiling for 30 min; roasting for 5 min in a pre-heated pan without oil; and drying using hot air at 80 °C for 15 h in the oven. Microwave heating was achieved by heating in a kitchen microwave. Nutrient analysis included determination of fat content using the Soxhlet method, and nitrogen content via the Kjeldahl method. Amino acids were determined via ion chromatography with a UV detection. The fatty acid profile was analysed by GC-FID, acrylamide was determined by UHPLC-ESI-MS/MS analysis. Our results indicated that both the methods of killing and culinary treatment influenced the nutritional value of *Tenebrio molitor* and *Gryllus assimilis*. Blanched and boiled mealworm larvae contained the lowest level of protein (54.76 g/100 g DM), and the highest content of fat (35.28 g/100 g DM), while blanched, oven-dried crickets yielded the highest levels of protein (72.95 g/100 g DM) and the lowest levels of fat (13.31 g/100 g DM). Culinary treatments uniformly resulted in increased proportions of PUFAs, while the percentage of SFAs exhibited a decrease. In contrast to lipids, the protein amino acid composition and quality, assessed via the essential amino acid index, as well as the profile of volatile compounds remained almost unchanged regardless of culinary treatment type. Safe levels of acrylamide (0.02-0.13 μg/g DM) were observed in treated samples. Our data may be helpful to nutritionists engaged in calculating the composition and energy value of foods. Given the rarity of raw insect consumption, gaining insights into the nutritional content of prepared insect-based dishes is of paramount importance. Supported by MEYS, LM2023064, GAČR, INPROFF, 21-47159L, and NAZV, QK23020101.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Koji fermentation-driven changes in volatile and aroma profiles of food ingredients containing lesser mealworm

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Fermentation of edible insects opens unlimited opportunities to diversify the use of insects. In analogy to miso paste preparation, the effect of koji fermentation with various materials derived from lesser mealworm (*Alphitobius diaperinus*) on volatile and aroma profiles was studied. (1) Fermentation of insoluble protein-rich insect material with black koji (*Aspergillus luchuensis*) and brown koji (*Aspergillus oryzae*) rice for 2 weeks led to the formation of a number of volatiles. Acetic acid, ethanol, and ethyl acetate were the most abundant volatiles in brown koji and commercial white miso samples. Sensory evaluation (0-100 scale) of insect fermented samples determined the dominant aroma attributes as "fermentation" (45-55) and "savory" (37-48). Insect samples fermented with brown koji received a higher aroma-liking score (41-48) than those with black koji (32-42). (2) Whole lesser mealworm powder combined with soybean was incubated for 2 weeks at different ratios (0-50%) with rice previously inoculated with brown koji. GC-MS analysis detected volatile compounds belonging to alcohols, aldehydes and acids; the attributes of the major volatiles being "greeny, fatty, fruity, sweet". A sensory test was conducted to demonstrate the applicability of the fermented material as a base for miso soup. The major aroma attribute of miso soup with fermented insect paste was "savory". The liking of miso soup decreased significantly with presence of insect powder. The maximum recommended addition was 25% lesser mealworm powder along with other ingredients such as koji rice and soybeans. The results also discuss the changes in pH, colour, and composition during the fermentation studies. In conclusion, koji fermentation can be used to modify aroma and volatile profiles of lesser mealworm-delivered food ingredients showing its potential for insect-based product development.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Innovative entomological solutions for dietary supplements

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The BEWARE INSECTA project focuses on exploring *Tenebrio molitor* as a novel protein source for dietary supplements, with the goal of optimizing rearing parameters impacting insect flour composition to create a protein-rich supplement meeting specific needs of both the elderly and athletes. Mealworms are reared in plastic trays (600 × 400 × 145mm) at 27 °C and 60% RH. Locally produced organic wheat bran (WB) is used as basic substrate, with agar (25 g/L) as water source. Two test substrates and a control substrate were assessed in triplicates from the 2nd month of larval development, added at two distinct times to limit volumetric density changes, as suggested by Deruytter and Coudron (2021): 80 mg and 100 mg per larvae at 4-w.o. and 8-w.o., respectively. Diets tested: Ctrl (WB), Diet A (WB, pea protein hydrolysate), Diet B (WB, pea protein hydrolysate, buttermilk powder). Their compositions (P,G,L) are respectively 16,65,2; 29,53,4; 26,55,6. Larvae from on-site breeding were transferred at a density of 6.7 larvae/cm^2 into tanks with test substrates. Larvae were harvested after 80 days of growth. Survival rate and average weight were calculated by weighting 50 larvae per tray. Protein content was measured with the Kjeldahl method. Survival rates were 90%, 86% and 93%, with average weights and protein contents of 83 mg (16.3% protein), 63 mg (15.1% protein), 69 mg (17.3% protein) for Ctrl, A and B diets respectively. Despite similar protein intake, group B exhibited better protein conversion (6.2% vs 4.6%) and higher weight gain (1.69 vs 1.41 mg/day). Protein content was correlated to P:C ratio (R^2 = 0.98). A variability in the results was observed that needs to be further investigation. Future steps include the dosage of proteins and amino acids in larvae fed various substrates, with a focus on fats content. INSECTA aims to establish specific, stable nutritional specifications for insect flour production, with adapted protocols for nutritional analyses in the insect matrix. Deruytter, D., Coudron, C.L., 2021. The effects of density on the growth, survival and feed conversion of *Tenebrio molitor* larvae. Journal of Insects as Food and Feed 8: 141-146.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Nutritional quality of *Tenebrio molitor* proteins obtained by Osborne fractionation

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The consumer acceptance of insects as food can be increased by processing, e.g. by isolating the proteins as functional ingredients. Therefore, the aim of our work was an extraction of mealworm (*T. molitor*) proteins following the example of Osborne fractionation with subsequent evaluation of the biological value of the protein isolates. A fractionation method, yielding four protein isolates based on their solubility, was optimized. Amino acid composition was determined using three different methods. For tryptophan, hydrolysis was performed under alkaline conditions with barium hydroxide followed by quantification using RP-HPLC-UV and internal standard N-α-methyltryptophan. The sulfurcontaining amino acids were determined by amino acid analysis (AAA) after oxidation followed by hydrochloric acid hydrolysis using cation exchange chromatography and ninhydrin post-column derivatization. The remaining proteinogenic amino acids were quantified after hydrochloric acid hydrolysis followed by AAA. The protein contents of the fractions and the protein yields were calculated from the sum of the total amino acids. For classification of biological value, the sum of indispensable amino acids (IAAs) was determined, and limiting amino acids with associated amino acid scores (AAS) were calculated in comparison to WHO recommendations. The most yielding fractions were the alkaline-derived fraction with 29%, and the aqueous fraction with 16%. Only 14% of the proteins remained in the insoluble residue. The biological value was assessed for the defatted insect meal, as well as for the two fractions with the highest yield. The aqueous extracted fraction showed the best values with an AAS of 1.21 limited by the sum of methionine and cysteine (560 mg IAAs/g protein). The alkaline extracted fraction and the meal follow with AAS of 1,13 and 1,12 (571/ 560 mg IAAs/mg protein), each limited by the sum of cysteine and methionine. Thus, mealworms and their protein isolates were shown to have a high nutritional quality comparable to conventional animal proteins, which is limited by sulfur-containing amino acids. Osborne fractionation is a suitable tool to obtain defined protein isolates for further applications.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Silkworm species *Bombyx mori* and *Samia ricini* as potential novel foods in the European Union

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More than 2,000 insect species are used for entomophagy worldwide. 17% of these belong to the order Lepidoptera, which is the second most important order of edible insects after Coleoptera. However, no Lepidoptera species has yet been approved as an edible insect under the Novel Food regulation in the European Union. This study investigates the potential of the silkworm species *Bombyx mori* and *Samia ricini* as novel foods for the EU market. The silkworms were reared on different feed sources, including fresh leaves, fresh vegetables, and agricultural side-streams. Artificial diets based on dried leaves were developed and processing methods, including drying, defatting, and extrusion, were adapted for Lepidoptera species. Food prototypes from larvae and pupae of the respective insects were produced and evaluated sensorially. Furthermore, the nutritional values of the pupae of *B. mori* and *S. ricini* were compared with those of the already approved insect *Tenebrio molitor*. *S. ricini* could be reared on local plant materials and agricultural side-streams such as carrot peels. The amino acid profiles of the respective silkworm species showed a high biological value with 1.8 g/100 g dry matter methionine in comparison to 0.7 g/100 g dry matter in mealworm larvae. Different types of bakery products, such as bread and extruded snacks, produced from the silkworm proteins received high sensory acceptance. The results allow the conclusion that the silkworm species *B. Mori* and *S. ricini* provide valuable nutrients for human nutrition. However, automated processes need to be developed for economic large-scale production.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Accelerating insect farming and consumption for health and livelihoods in Africa

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Scaling up insect farming for food in Africa has untapped potential to supply sustainable animal protein and important micronutrients to challenged food systems. Through novel value chains and pathways, it can contribute to improving nutrition, health and livelihoods. Despite these promises and a decade of piloting efforts, the intensity of insect farming for food remains modest or low across the continent. We investigate drivers for insect farming in a multi-site Randomised Controlled Trial (RCT) study in Kenya, Uganda and Ghana. Method: In a three-arm RCT design, implemented in 99 villages per country, we investigate effects on adoption of insect farming through (1) providing start-up farming and training package, or (2) combining the package with nutrition education and demonstration, motivating the use of insects in children's diet. Three small-scale farming systems are introduced, selected for species with a history of consumption in each country: cricket (*Acheta domesticus*) in Kenya; grasshopper (*Ruspolia differens*) in Uganda; and palm weevil larvae (*Rhynchophorus phoenicis*) in Ghana. The one-year implementation has been completed at all sites in early 2024. Results: At baseline (n = 8886 household heads), about 60% expressed willingness to take up insect farming. Around 3,000 households implemented the offered farming technology. Endline data will report on the experience, actual adoption and willingness to farm in the future, supporting targeted policies to accelerate insect farming in Africa. Conducted by the HEALTHYNSECT consortium representing Univ Energy and Natural Resources, Ghana; Makerere Univ, Uganda; Jaramogi Oginga Odinga Univ Science and Technology, Kenya; Jomo Kenyatta Univ Agriculture and Technology, Kenya; Masinde Muliro Univ Science and Technology, Kenya; icipe, Kenya; Dep Food Resource Economics (IFRO), Dep Plant and Environmental Sciences (PLEN), University of Copenhagen, Denmark. Supported by Danida, Denmark (grant 19-08-KU).

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Effect of crickets enriched complementary porridge and nutrition education on infant and child growth in Kenya: RCT

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Child malnutrition is a complex health state driven by poor diet quality and feeding practices. In alleviating malnutrition, insect is of interest due to their high-quality protein, rich minerals and vitamins. Potentially insect-based food are protein alternatives to dairy and flesh for infant transitioning from exclusive breastfeeding to complementary food (CF). Due to the challenging nature of infant nutrition in low-income settings, combination of nutrition-specific and nutrition-sensitive interventions are proposed for better outcome. We designed a 2 × 2 factorial randomized controlled trial with two embedded sub-studies: human milk intake (HMI) and gut health. To evaluate effect on baby's length gain, mothers of infants (6 months) were invited, infants screened for eligibility and enrolled between February and April 2023 at Rwambwa Sub-County Hospital, Siaya County, Kenya. A total 284 mother/infant dyads were randomly assigned to 4 study arms: control arm, cricket enriched nutrition (CEN) arm, nutrition education (NE) arm and a combined NE with CEN arm. The CF study products was provided as take-home infants daily serving, for 30 days monthly with rations adjusted for age repeated for 8 months between February 2023 to January 2024. The NE sessions were monthly personalized audio-visual sessions with education messages and appointment reminder sent regularly to mother's mobile phones. Infant anthropometric was measured using standard methods to asses growth monthly. Subsamples were assessed for HMI [n = 65] and gut health [n = 75]. Questionnaires on household characteristics, feeding practices and child health were built on KoboCollect App. Baseline infants were enrolled at 6.3 ± 0.5 months, weighed 7.6 ± 1.7 kgs with length 65.7 ± 3.1 cm and a malnutrition prevalence of 16.6%. At endline 244 dyads were seen recording 85.9% retention. Data analysis is on-going with results expected end of March 2024.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

University student perceptions of mealworm-based foods in New England, USA

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Early adopters of edible insects are likely young, progressive, flexitarian eaters who are environment and health-conscious; and interested in reducing their consumption of meat. University students in New England match this profile and increasingly demand diverse options for sustainable protein. This research used focus group sessions to investigate students' protein consumption motivations, opinions on novel alternative proteins, and sensory perceptions of insect-based foods. Undergraduate students (43) participated in 9 paid focus groups, during which they sampled 5 mealworm-based foods – falafel, granola, hard pretzels, and two types of cookies. Students preferred falafel and granola not only for flavour, but also for their potential as the main protein components in meals. The cookies and pretzel were well-liked but were viewed either as snacks or as a vehicle to help consumers overcome aversion. However, students expressed a strong interest in the mealworm flour used in these baked goods. In comparison to other alternative proteins like imitation meat, many students saw insects as less processed and more natural or transparent. Vegan and vegetarian students revealed that many of their ethical motivations against eating meat - such as animal welfare and environmental sustainability - also motivate them to eat insects. The findings suggest that this audience is amenable to entomophagy, and acceptance can be further encouraged by contextualizing insects to align with diet motivations.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Public acceptance of novel foods: lessons from potato

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Entomophagy can both improve human nutrition and increase sustainability of agricultural production. However, it is not entirely culturally acceptable to large segments of the human population, especially people of European descent. The history of potato, *Solanum tuberosum*, can simultaneously serve as a lesson on challenges of introducing novel foods to reluctant consumers and as an inspiring example on the ultimate success of doing so. Globally, potato is currently the fourth largest staple crop. While its share in people's diets varies around the world, it is pretty much universally accepted by consumers in all regions and from all cultures. Therefore, it is hard to believe that potato's initial spread from its centre of origin in the Andean region in South America received rather unenthusiastic reception in other areas. Yet, that was exactly what happened in the not-so-distant past. Potato was originally brought to Europe as a botanical curiosity. Although its potential as a food plant was recognized by some members of the educated classes, that idea was met with considerable suspicion by most of the population. Potato's road towards becoming a significant part of a common meal was long and difficult, and often required government sponsorship and extensive outreach. Once it was established as a staple food, however, potato dramatically improved people's nutrition, particularly for the socioeconomically disadvantaged strata of the population. There are good reasons to believe that edible insect entry into the agricultural and culinary mainstream may follow a similar trajectory.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Nutritional potential and mapping the landscape of the Korean edible insect industry

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South Korea has a historical proclivity for utilizing insects in both culinary and traditional medicinal practices. Silkworm pupae, locally referred to as *boendaegi*, hold a distinguished status as a gastronomic delicacy. While additional edible insects such as crickets and grasshoppers are acknowledged, the scope of commercial production predominantly was centred on *boendaegi*. Over the past decade, the registration of ten edible insect species has transpired. Among these, the honey bee drone is the latest addition to the list of edible insects. Our investigations, conducted using standard methodologies outlined by AOAC, have demonstrated the nutritional potential of these insects. There is a discernible surge in the prevalence of edible insect farms, 2,318 as of 2018, concomitant with an expansion in product diversity. There are five centres in the insect industry, namely Gyeongsangnam, Gyeongsangbuk, Daejeon, Jeonnam, Gyounggi, along with three insect breeding centres located in Gyeongbuk, Kangwon, and Chungbuk. Farms are mainly situated in rural areas. South Korea is among the countries authorized by EU regulations to export insects into the EU. Automated smart factories for insect processing are emerging and advancing. The product range extends from ingredients derived from edible insects to various industries, such as cosmetics, food, functional materials, fertilizers, and animal feed. The regulatory landscape has responded to this burgeoning industry, with formulated policies addressing the utilization of edible insects for both human consumption and animal feed. This paper endeavours to present a contemporary overview of the Korean edible insect industry, emphasizing the nutritional attributes of some prospective edible species, current industrial production dynamics, prevailing policies, and product diversification.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Unveiling the potential of black soldier fly larvae (BSFL) oil as a sustainable source of future food ingredients

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The world population is predicted to increase to 9.8 billion by 2050. The growing population and limited food supply lead to food insecurity and world hunger. To meet the global demand, food production will need to be increased by 70% by 2050. The food industry is searching for alternative, reliable, and sustainable solutions. Incorporating insect-derived components into food products shows a growing interest as most explored novel foods are not nutritious, healthier, and sustainable at once. Black soldier fly larvae (BSFL) is a rich source of nutrients, and traditionally consumed by several cultures around the world. Researchers have mainly focused on BSFL protein, and oil remains as a by product from protein extraction. This study was intended to explore the fatty acid profile and DPPH radical scavenging activity of BSFL oil compared to commercially available coconut oil (CO), a commonly consumed edible oil. Dried, powdered, Australian grown BSFL was rich in protein (54 ± 0.2%), oil (35 ± 0.7%), and essential minerals (Ca, Zn, Fe). BSFL oil was obtained under Soxhlet extraction and petroleum ether was used as the extraction solvent. Fatty acids were analysed by using Gas Chromatography equipped with Mass Spectrophotometer (GC-MS). BSFL oil showed significantly higher DPPH radical scavenging activity (64 ± 0.5%) compared to CO (52 ± 1.4%) ($P < 0.05$). Both samples were rich in medium-chain fatty acids (MCFAs) with a comparable fatty acid composition. Lauric acid, a MCFA with good bioactive and nutritional properties was observed as the most abundant fatty acid. BSFL oil contained 40 ± 0.1% of lauric acid followed by palmitic acid (22 ± 0.2%) and oleic acid (12 ± 0.1%) while CO contained 48 ± 0.7% of lauric acid. Total monounsaturated fatty acids (MUFA) content in BSFL oil (17 ± 0.1%) was significantly higher compared to CO (5.8 ± 0.6%) ($P < 0.05$). Overall, BSFL oil could be considered as a potential future food ingredient as it offers a healthier fatty acid profile and a good oxidative stability.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Eat bugs, not buzz: cultivating entomophagy through flavourful encounters

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Despite being widely embraced in many regions, edible insects encounter challenges in gaining acceptance in Western societies, primarily due to unfamiliarity and the associated disgust factor. The traditional promotional approach for entomophagy has emphasized nutritional values and environmental benefits. We propose a shift towards a hedonic-based campaign, leveraging insect tasting to overcome these barriers. Our strategy involved hosting tasting demonstrations, featuring various edible insect species, prepared in both visible and invisible forms by an experienced chef. Participants reported positive tasting experiences, with ”interested” being the most common reaction (65%). Individuals new to insect consumption were more associated with feelings of being ”educated” (56% vs 22%) and ”surprised” (56% vs 16%) than those who had consumed insects before ($P < 0.05$). After the event, participants, especially those who had not consumed insects before, showed a trend of increased willingness to eat insects, with their score rising from 3.13 to 3.68 ($P = 0.104$). While the tasting demonstration was well-received by all participants, those with prior experiences in insect consumption showed significantly higher willingness scores afterward (4.31 vs 3.68, $P < 0.05$). The result suggests that conducting tasting demonstrations is an effective intervention for familiarizing consumers with edible insects and improving their acceptance of such products. Nevertheless, it may be necessary to provide repeated exposure to further solidify the newly acquired familiarity. The study emphasizes the importance of collaborative efforts with culinary arts to acquaint consumers with crucial factors influencing acceptance of edible insects – taste, creativity, and enjoyment. By emphasizing the sensory appeal, we challenge the prevailing perception that insect consumption is solely driven by nutrition and sustainability considerations, fostering a more positive consumer outlook.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Protein quality of insects in view of methods applied

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Protein quality of insects in view of methods applied Insects are alternative protein sources with potential to diversify and sustain diets, but assessing their protein quality is complex due to their diversity. However, assessing protein quality of insects is necessary to fully recognize their potential and ensure optimal future applications. This review aimed to provide an overview of suitable methods to evaluate protein quality of insects. This is a concise review based on the available study on insect protein quality. While direct human studies are ideal to determine the protein quality of any food, ethical constraints often lead to use of animal models. Various methods, including digestible indispensable amino acid score (DIAAS), have been used to evaluate protein quality of some insect species, but discrepancies exist, partly due to chitin content not being accounted for in standard calculations. Use of standard nitrogen-to-protein conversion factor (NP) of 6.25 may overestimate protein content of insects. Several studies suggest insect-specific NP to mitigate this issue, impacting calculated DIAAS. Adjusting NP values to consider chitin is essential for accurate protein quality assessment of insects using DIAAS. Limited data on protein quality of insect suggest their high quality for human consumption, yet cultural acceptance remains a challenge. Scientifically substantiated arguments regarding insect protein's nutritional value compared to conventional proteins can aid in overcoming barriers. In conclusion, while the DIAAS method offers accurate protein quality representation, reconsidering NP calculations to account for chitin is necessary. Systematic assessment of protein quality across insect species is recommended to inform consumers and promote insect consumption as a sustainable alternative in human diets. Additionally, while farmed insect species show promise as alternative protein sources, assessing the protein quality of wild-harvested species is essential. Lastly, addressing challenges in insect protein quality evaluation and promoting their nutritional benefits can facilitate their acceptance as viable alternatives to traditional proteins.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Exploring on the potential of cricket protein hydrolysate in combating Alzheimer's Disease

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The nutritional and therapeutic potential of insect-derived proteins is increasingly recognized in scientific research. There is a growing interest in edible insects with a focus on protein hydrolysates derived from the cricket (*Acheta domestica*). These hydrolysates are considered potential functional food ingredients due to their high protein content and unique bioactive properties, especially their anti-inflammatory capabilities. In preparing the cricket protein hydrolysate (CYP), we used 7.5 Alcalase 2.4L (an endoproteinase from *Bacillus licheniformis*, 2.4 AU/g) and Flavourzyme 1000L, achieving hydrolysis larger than 90% (measured by TNBS assay) In tests focusing on the inhibitory effects of CPY on Alzheimer's Disease (AD) related enzymes, we found that the hydrolysate significantly inhibits the activities of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and BACE-1. Data from a Drosophila model of AD provided compelling in vivo evidence of the hydrolysate's efficacy. Remarkably, AD flies administered with CPY exhibited a significant improvement in the climbing index. Additionally, the data showed a decrease in β-secretase activity and reduced levels of Aβ1-42 peptides, comparable to a standard AD drug, donepezil. Moreover, in vitro tests involving Raw 264.7 macrophages and BV2 microglial cells showed that pre-treatment with CPY significantly downregulated proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, and downregulated the expression of reactive oxygen species (ROS). This suggests that CPY may play a protective role in brain inflammation. In summary, our findings suggest that CPY could be a promising candidate for developing alternative therapeutic agents for AD, owing to its enhanced inhibitory effects on AD-related enzymes. This study bridges the gap between traditional insect protein usage and modern biomedical applications, offering insights into the potential of entomophagy in neurodegenerative disease management.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Feasibility of adult giant honey bee as a novel protein source through the development of fermented seasoning sauce

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Edible insects are known for their protein content and resource efficiency. However, no prior studies have explored using bees as a protein source for sauce production. This study used adult bees (*Apis dorsata* F.) to create fermented seasoning sauces through the soy sauce fermentation process with *Aspergillus oryzae* for bee koji formation. Two sauce samples (D431, bee: water: rice flour = 4:3:1 and D53, bee: water = 5:3) underwent 30-day fermentation in 23% brine at 30 °C. Colour measurement in the CIE L*a*b* colour space showed a significant difference in L values, with D53 having a higher L value and a correspondingly higher b value and lower a value than D431. The sample pH values decreased from 7.00 on day 0 to 5.07-5.13 on day 30, and a gradual decrease in total soluble solids and electrical conductivity was observed over the 30-day period. The protein composition of the D432 and D53 samples exhibited a significant increase to 1.69% and 2.04%, respectively, when compared to the control sample without fungal activity. Additionally, the total nitrogen content of the sauces increased to 0.307-0.337% by day 30, indicating the degradation of proteins by *A. oryzae*. A preference test using a 9-point hedonic scale showed no significant differences ($P < 0.05$) were observed for the attributes of colour and aroma between the samples. The overall taste of D53 was found to be significantly preferred ($P > 0.05$) by consumers. Principal Component Analysis results revealed distinct differences in physical, chemical, and nutritional quality among fermentation formulas. The research highlights the possibility of using adult bees as a high-protein food source.

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First online database of insects as food and feed

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Over the million species known, more than 1,600 species of 18 orders have been reported as edible insects (either directly as food, or as feed for livestock), of which 5 orders contain more than 100 different species. The great majority are directly harvested in nature though some species are farmed on a large scale. Beside the six most common commercially-available edible insect species such as cricket (*Acheta domesticus*), honeybee (*Apis mellifera*), domesticated silkworm (*Bombyx mori*), mopane caterpillar (*Imbrasia belina*), African palm weevil (*Rhynchoporus phoenicis*) and yellow meal worm (*Tenebrio molitor*), information regarding the biology, life history traits of other lesser-known species is hard to gather, and therefore time-consuming. For the first time, we introduce an accessible, web-based, database aimed at addressing this issue, offering comprehensive information on each species to maximize accessibility. The database was created using all available literature, and the taxonomy was corrected by expert taxonomists in each order, and will be updated regularly. For each species, distribution records, host plants, seasonality, stage consumed, life-history traits and occurrence in the literature will be resumed on a datasheet. We hope that this collaborative platform, displayed and accessible at the conference through a QR code, will stimulate research for the lesser-known diversity of edible insects.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Entoferritin: investigating iron binding insect proteins as a potential source of dietary iron

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Iron deficiency is worldwide a prevalent health concern, necessitating the exploration of sustainable iron sources. Edible insects, although relatively unexplored in this context, show promise due to their rich potential for bioavailable iron. Insect ferritin, or entoferritin, offers a novel perspective as an iron supplement. This literature review assesses the viability of entoferritin as an iron source, comparing it to mammalian and plant ferritins to delineate its distinctive properties. The results of this study show that entoferritin is a soluble protein complex with a strong affinity for iron. These distinctive features may allow for its efficient purification through commonly employed techniques such as size exclusion chromatography and metal affinity chromatography. These purification techniques can seamlessly integrate with existing edible insect processing chains, minimizing disruptions to other valuable insect-derived products. Moreover, the capacity of entoferritin to amass significant levels of bioavailable iron, which can be readily absorbed through human endocytosis mechanisms, presents an area of potential significance. However, understanding entoferritin's mechanisms of delivery into the human iron pool, as well as addressing issues related to bioavailability and safety, requires comprehensive exploration. In this communication we will show how to valorize entoferritin, addressing global iron deficiency concerns, in the frame of an integrated utilization of edible insects. Further research should focus on entoferritin's bioavailability, safety profile, and practical applications to fully leverage its potential for mitigating iron deficiency and promoting sustainable insect-based nutrition.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Techno-functional properties in binary blends of gluten-free flours and *Gryllus bimaculatus* powder

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Gryllus bimaculatus powder, a sustainable source of animal protein can be a suitable ingredient for formulating enriched gluten-free products. This study investigated the impact of substituting rice, maize, proso millet and finger millet flours with 5% , 10%, 15%, and 20% *G. bimaculatus* powder on pasting and microstructural properties. Significant differences ($P \leq 0.05$) were observed in all pasting parameters except for peak time in proso millet blends. Pasting viscosities gradually reduced with *G. bimaculatus* powder addition: Average reductions in peak viscosity were in the order of rice (546.40 mPa*s), finger millet, maize and proso millet (224.40 mPa*s) blends, with greater reductions at 5% and 10% substitution levels. Positive setback and breakdown viscosities were higher in proso millet, followed by rice, finger millet and maize blends: The latter two blends formed heat-stable pastes suitable for porridge development. Average final viscosity reductions were in the order of rice (1045.00 mPa*s), maize, proso millet and finger millet (315.75 mPa*s) blends. Addition of *G. bimaculatus* powder reduced the peak time but increased pasting temperatures with up to 1.8 °C in rice blends and 13 °C in finger millet blends. Cryo-scanning electron micrographs of flour pastes revealed a regular, dense and continuous network of structures, which gradually degenerated into irregular structures and a discontinuous network upon the addition of *G. bimaculatus* powder. These observations suggest technological exploitation of *G. bimaculatus* powder in development of low viscosity and heat stable gluten-free products with enhanced nutritional quality, particularly with maize or finger millet flours.

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The contribution of edible insects to nutrition security in the Eastern D.R. Congo

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Edible insects are perceived as an incredible opportunity in mitigating the major challenge of sustainably producing healthy foods for a growing world population, in the face of climate change uncertainties over the coming decade. This study aimed at assessing the nutrient composition and sensory properties of *Acheta domesticus*, *Apis mellifera*, *Gnathocera trivittata*, *Gryllotalpa africana*, *Imbrasia epimethea*, *Imbrasia oyemensis*, *Locusta migratoria*, *Macrotermes subhylanus*, *Nomadacris septemfasciata*, *Rhyncophorus phoenicis*, *Ruspolia differens* and *Rhynchophorus ferrugineus* consumed in Eastern D. R. Congo. The investigated edible insects are highly nutritious with proteins (20.67-43.93 g/100 g) and fats (14.53-36.02 g/100 g) being the major macro-nutrients with an incredible appreciation and satisfactorily energy (422-534.08 Kcal) proving their potential in improving diets and preventing undernutrition in countries which are plagued by micronutrient deficiencies. The high potassium (24-386.67 mg/100 g), sodium (152-257.82 mg/100 g), magnesium (32-64 mg/100 g), iron (5.3-16.13 mg/100 g), calcium (25-156.67 mg/100 g) and zinc (11-19.67 mg/100 g) content make the assessed edible insects a useful mineral-containing ingredient for food enrichment. A scatter plot of matrices (SPLOM) and Pearson's correlations between sensory attributes and nutritional composition showed a negative correlation ($r = -0.45$) between protein and appearance. While no strong correlation was observed between nutritional attributes and sensory acceptance, a positive correlation was observed between potassium and aroma ($r = 0.50$), after taste ($r = 0.50$) and acceptability ($r = 0.52$).

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Macrotermes subhylanus flour inclusion in biscuits: nutritional, sensorial and microbial properties

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Edible insects have been suggested as a food source that could address economic, environmental, and health concerns as the population expands. This study aimed to create a novel biscuit by adding *Macrotermes subhylanus* flour to whole wheat flour in various concentrations (0, 5, 10, 15 and 20%). The moisture content of the composite flours ranged from 6.8 to 7.7%, while that of the biscuits ranged from 2.8 to 7.9%. A notable significant difference ($P < 0.05$) was observed in the protein content of the composite flour and biscuits as the insect flour concentration increased, ranging from 15.0 to 21.5% and 17.3 to 20.6%, respectively. The lightness of the composite flours decreased significantly ($P < 0.05$) with an increase in edible insect flour addition. At the same time, the colour attributes of redness and yellowness did not exhibit any statistical differences ($P > 0.05$). Biscuits generally showed substantially lower L*, indicating that they were darker in colour than the corresponding composite flours. The water activity of the biscuits ranged from 0.4 to 0.6. In this study, Bacillus cereus, total coliforms, *E. coli* and *Salmonella* spp were not detected. Sensory evaluation revealed that the substitution level (up to 20%) is ideal for preparing acceptable insect-based biscuits. *Macrotermes subhylanus* flour could be used as a novel functional ingredient in the food industry.

INSECTS AS FOOD, PRODUCT DEVELOPMENT AND CONSUMER ACCEPTANCE

Willingness among Taiwanese consumers to eat insects directly as food or indirectly as animal feed

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Factors affecting willingness to eat insects have been studied predominantly in Europe and North America, or countries like Thailand with traditional entomophagy. Studies typically focused on consumer attributes such as levels of food neophobia or disgust, and the use of "disguise" by presenting insects in the form of a normally grain-based food item. This talk covers surveys examining attitudes in Taiwan, an Asian culture where edible insects are not commonly consumed. Qualtrics software was used to survey students from Taiwan's largest university. One survey correlated attitudes to entomophagy using the Entomophagy Attitudes Questionnaire (EAQ) and food neophobia to the desire, intention, and probability of actually eating insects within the next month, identifying factors affecting passive rejection from lack of supply instead of active rejection while validating the EAQ in Taiwan. While media and academic attention to insect bioconversion of waste into animal feed is ever increasing, research on consumer acceptance thereof globally has been sorely lacking. Thus, a second survey incorporated the validated EAQ, randomized exposure to informational material on the environmental benefits and/or nutritional equivalency of animal feed made from insects as alternatives to soymeal and fishmeal, and a willingness-to-pay (WTP) survey comparing meat and eggs from animals fed insects as feed to those fed standard diet. The use of informative texts to try and influence consumer responses was to see if education and messaging can overcome pre-existing hesitance to insect consumption. Finally, a third survey used the EAQ and informative texts with WTP comparisons of "disguised" insect products with traditional "disguised" meat products in local cuisine, such as fish and shrimp balls. Strongly neophobic and entomophobic consumers may remain unwilling to add insects to their diets even if they are educated on the rationale for doing so, leading to a negative willingness to pay for insect-based foods, but indirect consumption of insects through animals fed them as feed is expected to be far more acceptable to the general populace.

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Insect-based dinner meals for meat replacement: insights from SUSINCHAIN

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Edible insects are a promising alternative protein source for more sustainable diets. However, they are primarily used in snacks or adventure foods rather than as main meal components. To significantly reduce meat consumption and promote environmentally sustainable diets, insects must transition into key ingredients in main dishes and serve as meat substitutes. Six unique insect-based dinner products were developed and produced by SUSINCHAIN project partners. A randomized control trial was conducted in which families were provided with either three insect-based or plant-based dinner meals weekly for six weeks, with the aim of substituting 20% of their meat protein intake. The objective of this study was to investigate the impact of exposing Danish families (an adult and 8-10 year old child) to insect-based or plant-based dinner menus on dietary pattern, meat intake, and protein intake over a six-week intervention period. Participants maintained their estimated daily total protein intake, while reducing daily meat protein intake. In the insect-based menu group, adults and children reached an average 5.5% and 2.3% weekly meat replacement, respectively. In the plant-based menu group, adults and children replaced 9.0% and 4.3%, respectively. Meat attachment was the only psychosocial variable that had a significant effect. Product type is crucial for meat substitution; some products, despite their high sensory appeal, were not seen as meat replacements. Balancing insect protein with sensory appeal posed challenges, emphasizing the importance of portion size and presentation. The insect-based foods faced more barriers than the plant-based, indicating a demand for more palatable options. Achieving notable meat protein replacement requires an integrated approach that combines product development and an understanding of consumer preferences.

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Effect of enzymatic hydrolysis on the allergenicity and biofunctionality of edible insect proteins: a review

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Insects are an alternative source of future food which continues to be developed as a source of protein or are known as protein-based insects. However, some people do not accept insects as food because they are afraid of allergenic compounds and they have not experienced their biofunctional benefits. Therefore, this research aims to reveal the effect of the enzymatic protein hydrolysis process on the allergenicity and biofunctionality of edible insects. The method used in this research is a systematic literature review. We have selected 107 out of 1,096 articles sourced from scientific journals indexed by trusted electronic databases. The results of our study report that processing insects using proteolysis or enzymatic hydrolysis can be an effective solution to produce hypoallergenic insect protein hydrolyzate powder. The use of protease enzymes determines the structure of the oligopeptide formed and the combination of protease enzymes can produce unique bioactive peptides. The temperature and length of time of the hydrolysis process influence the yield of the protein produced. Another potential of breaking down the protein structure is to produce bioactive peptide compounds which have biofunctionalities such as antioxidant, anti-inflammatory, antihypertensive, anticancer, antidiabetic and antimicrobial.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Assessment of probiotics from black soldier fly Larvae on the performance, immunity, and gut health of broiler chickens

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Black soldier fly larvae (BSFL) are reported to contain lactic acid bacteria (LAB) species and strains that have the potential to function as probiotics. This study aimed to evaluate the effects of BSFL probiotic on performance, gut health, and immune response of broilers. Herein, 250 broilers day old chick were divided into 5 treatments with 5 replicates. The probiotics used are produced from fermentation of BSFL extract. This study used a completely randomized design by adding probiotics to drinking water, where the treatments were: T0 = without probiotic (control), T1 = commercial probiotic 1 g/l, T2 = BSFL probiotic 1 ml/l, T3 = BSFL probiotic 3 ml/l, T4 = BSFL probiotic 5 ml/l. The broilers get the same diet in each treatment. The variables observed were growth performance, weight of internal organs, population of microorganisms in the digestive tract, intestinal histopathology, blood hematology, and immune response. The results showed that the addition of BSFL probiotics at the highest dose could significantly increase ($P < 0.05$) the BWG and FBW of broilers, which are 170 g and 249 g higher than broilers reared without using probiotic and commercial probiotic, respectively. Population of *Bacillus* sp. and LAB with the addition of BSFL probiotic up to 5 ml/l were mostly located in the intestines of broilers which ranges from 10^5-10^9 CFU/ml. The addition of BSFL probiotics at different doses can decrease ($P < 0.05$) of lymphocytes, heterophils, and heterophils/lymphocytes ratio. The immune response of broilers showed that increasing BSFL probiotics ($P < 0.05$) increased immunoglobulin G and decreased immunoglobulin M. The addition of BSFL probiotics did not show a significant effect on the weight of internal organs and intestinal histopathology of broilers. On the other hand, the amino acid content derived from BSFL fermentation can influence the results beyond the inoculant effect. In conclusion, BSFL derived probiotics can be applied to broilers as a productive and functional symbiont in improving growth performance, immune response and have no negative impact on gut health.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Growth performance, nutrient digestibility and oxidative stress in weaned piglets fed black soldier fly meal

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Hermetia illucens (HI) meal has already been tested in pigs, but no data is currently available under commercial conditions. This study investigated the effects of HI meal (as replacement of soybean and fish meals) on growth performance, nutrient digestibility, and oxidative stress (for its potential antioxidant properties) of weaned piglets. A total of 336 28-day-old, weaned piglets (6.2 ± 0.5 kg) were allotted to 4 diets (4 boxes/diet, 21 piglets/box, 3 feeding phases [I, II and III]): C (control), HI3 (3% HI meal), HI6 (6% HI meal), and HI9 (9% HI meal). Growth performance and faecal nutrient apparent digestibility coefficients (ADCs) were calculated at the end of each feeding phase (day 13, 29 and 63), and blood oxidative stress (dROMs and OXY-Adsorbent Test) was investigated at the beginning and end of the trial. Data were analysed by SPSS software ($P \leq 0.05$). In phase I, HI9 piglets displayed higher feed conversion ratio (FCR) than C ($P < 0.05$), while higher average daily gain (ADG) was detected in HI9 animals than C and HI3 in phase II ($P < 0.05$) – along with HI6 and HI9 piglets showing lower FCR than HI3 ($P < 0.05$). In phase III, lower FCR was highlighted in HI3 and HI9 groups than C ($P < 0.05$). The overall growth performance were, however, not influenced by dietary HI meal inclusion ($P < 0.05$). In parallel, HI6 and HI9 piglets displayed higher crude protein (CP, phase I; $P < 0.01$) and ether extract (EE, phase I and II; $P < 0.001$) ADCs than the other groups, with higher dry matter (DM) ADC being also identified in HI6 animals than C and HI3 (phase I; $P < 0.05$). In phase III, C and HI9 groups showed higher DM (C; $P = 0.001$), CP (C and HI9; $P < 0.001$) and EE (HI9; $P < 0.001$) ADCs than the other piglets. Higher OXY values were highlighted in HI6 and HI9 than C ($P < 0.05$). In conclusion, increasing inclusion levels of HI meal may improve growth performance and nutrient digestibility – as well as stimulating an antioxidant response – in weaned piglets. Supported by Agritech National Research Center (PNRR CN00000022).

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Hermetia illucens oil in the diet of dairy cows: does it affect feed intake, milk yield and milk main constituents?

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The limited availability and sustainability concerns on the use of conventional feed sources in ruminant nutrition have led researchers to look for new sustainable feedstuffs, among which we find insect oil. In this study, we evaluated the effects of replacing palm oil with *Hermetia illucens* (HI) oil in dairy cow diet on feed intake, milk yield and milk main constituents. Twenty-six Valdostana cows were divided into two groups and fed hay *ad libitum* and isonitrogenous and isoenergetic concentrates containing palm oil (PO group) or HI oil (HI group) at 3% as fed. The PO concentrate was rich in C16:0, C18:2 n-6, C18:0 and C18:1 *c*9 (16.7, 13.6, 10.5 and 5.6 g/kg DM, respectively), while the HI one was rich in C18:2 n-6, C16:0, C18:1 *c*9 and C12:0 (17.5, 8.9, 8.0 and 4.9 g/kg DM). After a 2-week diet adaptation, individual feed intake and milk yield were recorded daily for 50 days. Every 10 days, milk samples were analysed for fat, protein, casein, lactose, and urea contents (MilkoScan™ 7 RM, Foss Electric, Hillerød, Denmark). Data were statistically analysed as a repeated measure design, considering the effects of diet, time, and their interaction. The dietary treatment did not modify ($P > 0.05$) feed intake (15.5 vs 15.6 kg DM for PO and HI groups, respectively) and milk yield (19.8 vs 20.2 kg/head × d). The contents of milk main constituents (fat: 38. 1 vs 38.6 g/kg; protein: 30.4 vs 30.7 g/kg; casein: 24.2 vs 24.4 g/kg; lactose: 47.9 vs 47.5 g/kg; urea: 19.2 vs 19.3 mg/dL) remained unaffected by treatment. The obtained results suggest that HI oil, which is locally produced in Europe unlike extra-EU imported palm oil, does not exert any negative impact on feed palatability and milk production performance, and it can safely replace palm oil in cattle nutrition. (Supported by Green ERA-Hub HiGa project).

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Effects of dietary black soldier fly larvae meal inclusion on the growth performance of silkie crossbreed chicken

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Black soldier fly larvae (BSFL) can be grown on a wide range of waste substrates, offering a sustainable alternative to soybean meal as an ingredient for animal feed. The high protein and fat contents and favourable amino acid and fatty acid profiles of BSFL meal contribute to making it a suitable ingredient in broiler diets. BSFL meal also contains bioactive components. However, the impact of dietary BSFL meal inclusion on the growth performance of slow-growing silkie crossbreed broilers has not been evaluated yet. Based on previous findings, we hypothesised that BSFL meal inclusion can increase feed efficiency (gain:feed; G:F) by increasing the average daily gain in silkie crossbreed chickens. During a three-week long feeding trial, 72 silkie crossbreed chickens of 39 days of age were randomly assigned to three diets: control diet containing 260 g/kg soybean meal (CON), and partial replacement of soybean meal with 50 g/kg (BSFL50) or 150 g/kg BSFL meal (BSFL150), respectively. The observational units were cages of 3 birds each and each treatment had 8 replicates. Weight gain, feed consumption and G:F were monitored weekly and treatment effects were evaluated by one-way ANOVA. Compared with CON, BSFL50 showed a trend of increased weight gain ($P = 0.06$), while BSFL150 increased weight gain significantly ($P = 0.04$). Feed intake was not affected by the treatments ($P = 0.84$). Compared with CON, BSFL50 showed a trend of increased G:F ($P = 0.06$), while G:F was increased in BSFL150 significantly ($P = 0.01$). Our findings indicated that partial replacement of soybean meal with BSFL meal can increase weight gain and feed efficiency in silkie crossbreed chicken. Our ongoing analyses will further evaluate intestinal health effects and economic aspects of dietary BSFL meal inclusion in silkie crossbreed chicken.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

The potential of insect meal as an alternative protein source in dry premium dog and cat food

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Insect meal (IM) is positioned as a sustainable alternative protein source to commodity proteins and is increasingly being used in pet food formulations. Despite the adoption of IM in pet food, there is limited published literature on the palatability by pets for IM-based products. In this study we compare palatability, preference and digestibility of an IM based pet food to a premium commercial pet food for both cats and dogs. Two levels of IM were added to a standard premium commercial dry dog (at 10% and 20% inclusion) and cat (at 5% and 10% inclusion) food ration. Three rations of 24% crude protein (CP) dry dog food and 30% CP dry cat food were formulated by replacing the poultry meal with IM. The design of the palatability and preference tests was a complete randomised design using 20 beagle dogs and 15 mongrel cats, over 10 days of testing and 7 days of resting. A replicated 3×3 Latin square design with 7 days wash out period and 15 days of sampling was used for digestibility coefficient determination, with 18 dogs and 15 cats. There was no significant difference for first choice frequency or first empty bowl frequency for dogs between the control and, 10% ($P > 0.58$), or 20% ($P > 0.27$) IM diets. But, dogs chose the 10% IM diet as their 1st choice ($P < 0.00$) and first empty bowl ($P < 0.05$) more frequently than the 20% IM diet. There was no significant difference for first choice frequency between control and, 5% ($P = 1.000$), or 10% ($P = 0.762$) IM diets for cats, but the 5% diet had the frequency of first empty bowl significantly more than the control diet. Cats chose the 10% IM diet as their first choice ($P < 0.012$) and 1st empty bowl ($P < 0.05$) significantly more frequently than the 5% diet. Digestibility results performed similarly to previously published studies and show better digestibility for all IM diets for dogs, but not significantly different for cats. In conclusion, cat and dog food formulations can include at least 10% and 20% inclusion, respectively, of IM without impacting palatability, while improving protein digestibility for dogs only.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Live black soldier fly larvae as dietary supplementation for laying hens: towards sustainability for high-quality eggs

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This study evaluated the effect of live black soldier fly larvae (BSFL) supplementation on egg quality. 108 Lohman hens were housed (16-34 weeks age) into 27 cages (9 replicates/treatment, 4 birds/pen), assigned to 3 groups: control (commercial diet) and 2 groups fed commercial diet plus 15% or 30% (on the expected daily feed intake (DFI), as fed basis) of live BSFL. Egg physical traits were assessed each month; eggs chemical composition, nuclear magnetic resonance (NMR) metabolites, yolk fatty acid (FA) profile were evaluated at the beginning, half, end of the trial. Data were analyzed with R software considering the effects of diet, time, their interaction. Live BSFL supplementation did not significantly affect egg physical traits, whereas the FA profile of eggs' yolk and the proportions of most FAs significantly changed. BSFL hens' eggs had higher saturated FA and polyunsaturated FA (PUFA, $P < 0.05$), lower monounsaturated FA ($P < 0.001$), increased rates of C18:2n6 ($P < 0.05$) and C18:3n3 compared to the control ones ($P < 0.001$), without significant differences in the ratio n-6 and n-3 PUFA. Hens' age impacted the eggs physical traits, chemical composition, metabolites of egg white and yolk, yolk FA profile ($P < 0.05$). A supplementation with live BSFL up to 30% on DFI may be safely used in laying hens feeding programs without impairing egg quality.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

From traditional to industrial use of insects as feed: a review

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Insects are a natural diet for fish and poultry species and to a lesser extent for pigs and dogs/cats. In traditional farming, poultry gather their food in a free roaming manner, and insects are part of the diet. Similarly, a large fraction of the diet of freshwater fish consists of aquatic insects. These features are exploited by farmers all over the world, considering the high costs of feed for production animals. For example, farmers lure termites to baskets filled with organic matter, and the insects are then fed to chickens. They also employ light sources above fishponds to attract insects for their fish. More sophisticated methods are, for example, attracting naturally occurring houseflies to containers filled with organic waste, often with manure. The captured maggots or pupae are then fed to poultry. We discuss the following insect groups as feed sources for poultry, pigs, fish and cats/dogs: bees, caterpillars, cockroaches, flies, grasshoppers and termites. Feed for poultry can also consist of insect pests, which are then controlled at the same time, for example ducks to control rice pests. Leftover from the silk industry can also be used to feed chickens, pigs and fish. Insects are also commonly used as bait for fishing. The interest for a more industrial production of insects such as the black soldier fly and housefly as animal feed started in the 1970s. Since about 15 years, large scale rearing of insects for animal feed has taken off, the industry receiving more than 1.5 billion of dollars in investments. The market is expanding, the legislation is becoming more conducive, while the academic interest is increasing exponentially. The environmental, nutritional and functional benefits of insects as feed are more and more recognized.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Effect of black soldier fly and lesser and yellow mealworm meal on growth performance and gut health of weaned piglets

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Insects such as black soldier fly larvae (BSFL), lesser mealworm (LMW), and yellow mealworm (YMW) are being explored as sustainable protein sources that could enhance the growth and gastrointestinal well-being of weaned piglets. This study aimed to assess the effect of BSFL, LMW, and YMW on growth performance and gut health parameters when fed to weaned piglets. Ninety-six crossbred (Landrace × Yorkshire) × Duroc) male weaned piglets, with an average weight of 6.6 ± 0.5 kg, were used. Over a two-week period, piglets were fed either a comprising 14% enzyme-treated soybean meal (CTRL) or diets containing 2.66% enzyme-treated soybean meal with defatted BSFL, LMW, and YMW at 8.35, 9.00 and 7.66%, respectively. Each diet group comprised 24 randomly allocated piglets housed individually. There were no significant differences observed among dietary treatments for growth performance parameters. Immunoglobulin A (IgA) levels were consistent across all treatments, while D-lactate concentration remained unaffected. Piglets fed LMW and YMW diets exhibited lower diamine oxidase (DAO) concentrations (321 and 309 ng/ml, respectively) compared to those on the CTRL diet (382 ng/ml, $P < 0.05$), indicating potential mucosa barrier function protection. Levels of Enterococci and Lactobacilli in digesta did not differ significantly among treatments. Lactic acid levels in small intestine varied between 48.6 (CTRL) and 63.4 (BSFL) g/100 g of total organic acid. Total short-chain fatty acid concentrations in colon digesta did not vary significantly among dietary treatments. In conclusion, incorporating defatted BSFL, LMW, and YMW into weaned piglet diets, showed similar effects on growth performance and most gut health parameters compared to the CTRL diet. Additionally, reduction in plasma DAO suggests potential benefits for intestinal mucosa barrier function with mealworm-inclusive diets.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Digestibility and performance of black soldier fly meal in Asian seabass (*Lates calcarifer*)

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Amidst the exploration of alternative aquafeed ingredients, insects are emerging as promising novel protein sources. Among these, the black soldier fly (*Hermetia illucens*) has shown remarkable efficiency in converting various organic materials and black soldier fly larvae meal (BSFM) for animal feed is on the rise. However, it remains unclear whether BSFM can fully replace all fishmeal in aquafeeds and what the potential consequences might be on digestibility, and digestive metabolism. This study integrates the findings from two trials conducted on Asian seabass (*Lates calcarifer*), with Trial 1 focusing on estimating the apparent digestibility coefficients (ADC) of BSFM in comparison to soy protein concentrate meal (SPCM), a well-known ingredient in aquafeed. In Trial 2, different levels of BSFM inclusion (5%, 10%, and 15%) were investigated as replacements for fish meal. Three experimental diets, all isoenergetic and isonitrogenous with yttrium markers, were formulated and randomly assigned to nine tanks, with three tanks per diet. Each tank housed 110 fish (90.37 g ± 12.88), which were hand-fed to satiation twice a day for 28 days. Faecal samples were collected periodically throughout the trial, starting seven days after the initial feeding with formulated diets. Linear decreases in growth and feed intake were observed with increasing levels of BSFM inclusion ($P < 0.05$). The feed conversion ratio (FCR) worsened with increasing BSFM inclusion compared to the control diet (0.97 ± 0.04) ($P < 0.05$), with values of 1.1 ± 0.0, 1.13 ± 0.02, and 1.3 ± 0.06 for the increasing BSFM inclusion diets. BSFM ADC was 85.3% for dry matter and 93.3% for protein. The impact of BSFM inclusion on gut enzyme activity and gut and brain transcriptomics analysis will be presented to explore the interaction between feed intake and fish performance.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Development of a feed supplement utilizing live black soldier fly larvae to improve the productivity of laying hens

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A feeding trial was conducted with 162 31-week-old Hy-Line Brown hens supplemented with live larvae of *H. illucens* for 24 weeks. The hens were distributed into three groups of 54 hens (03 replicates/ group). For egg productivity (weekly assessed) and egg quality (at the end of the study) were evaluated egg weight, breaking strength, shell thickness, albumen height, Haugh units, yolk index, yolk colour, and shell colour. This study included microbiological analysis through metagenomic sequencing of the hens' intestinal contents. At the end of the trial, fresh faeces were collected from the digestive tract of the hens. DNA was extracted, PCR product was sequenced for bacteria phylum identification. To monitor stress, corticosterone quantification was performed as a function of the type of feeding of the groups. We compared rates of corticosterone in the yolk of eggs laid at the end of the trial period when the hens had well-established behaviours. By assessing humoral immune response to an unexpected avian respiratory disease, antibody titters specific to APV, IBV, MG, and MS were determined at weeks 0 (18 hens), 8, 16, and 24 (27 hens). The purpose of the study was to assess the potential benefits, on a pilot scale, of incorporating this feed supplement. The results confirm that supplementation with live *H. illucens* larvae in the diet enhances egg productivity during the later stages of hen production and improves the nutritional quality of the eggs. Furthermore, this dietary modification influenced the composition of the hens' intestinal microbiota, promoting the presence of bacteria with characteristics beneficial for maintaining avian health.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Hermetia illucens larvae reared on food-waste to supplement fishmeal-limited diets in *Lates calcarifer*

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Black soldier fly larvae (BSFL) is a promising alternative to replacing fishmeal (FM) in Barramundi diets. However, past studies on FM replacement with BSFL in Barramundi were limited to (1) experimenting on fingerlings with BSFL, (2) using BSFL reared on agricultural waste and (3) experimental diets that did not account for potential discrepancies in micronutrient content. This study represents, for the first time, the viability of supplementing fishmeal-limited diets with BSFL reared on food waste and its effects on post-fingerling Barramundi. Four isonitrogenous and isocaloric diets were designed with varying amounts of FM and BSFL with two diets inclusive of additional amino acids (AA) (FM40%, FM20% + AA, FM20% + BSF20%, FM20% + BSF20% + AA). A 10-week feeding trial was conducted on 360 Barramundi (79.90 ± 9.44 g) randomly assigned into triplicated tanks containing 30 fish each, fed twice daily to satiety. A digestibility trial ensued to assess the apparent digestibility coefficients of crude protein and fat. Effects on fish immunology were assessed through immune gene expression and a scale drop disease virus challenge. Samples from each diet were randomly selected to analyze gut microbiomes, haematological parameters, histomorphological patterns in fish digestive systems and body chemical composition. Finally, we conducted a sensory testing of the fillets. Percentage weight gain and specific growth rates were higher in BSFL included diets. All experimental diets shown improvements to survival rates and feed conversion ratio compared to the control diet. Other analyses are still ongoing and will be discussed later.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly (*Hermetia illucens*) oil derived monoglycereides as a powerful antimicrobial feed additive

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Anti-microbial resistance (AMR) is a major health concern. Use of antibiotics in agriculture is implicated as a cause of AMR. However, this use is critical to maintain yields and retain low food prices. So, novel solutions are needed. NeoManna aims to answer this need with solutions based on circular economy, focusing on the black soldier fly (BSF). Our first product, NeoMAG™, is based on BSF oil. BSF oil is made primarily of triglycerides (TAG), has high contents of medium chain unsaturated fatty acids (FA, e.g. C12:0 and C16:0). The anti-bacterial activity on monoglycerides (MAG) of such FA, especially monolaurin (C12:0) is well documented. We developed a process transforming BSF TAG to MAG, resulting in >50% MAG and only 1% free FA. The FA profile was unchanged, with C12:0 as the major FA, i.e. monolaurin. We developed a powdery form of this MAG rich BSF oil, to add to livestock feed in commercial settings. NeoMAG™ secured registration in the EU and Israel and is in manufacturing and undergoing extensive field trials. Testing NeoMAG™ on broilers (n = 2,300 for test and control groups) at different dosing on top of the best available diet resulted in 2.92-3.6% higher marketing body weight (MBW), up to 5.2% higher feed conversion rate (FCR) and a 2.7-8.8% increase in production efficiency factor (PEF). Versus the best available diet (n = 33,500 for each), NeoMAG™ lead to 9.73% higher MBW, 2.31% higher FCR and 9.65% higher PEF. Testing NeoMAG™ vs. commercial feed on pre-starter swine (12 pens of 6 pigs each for each treatment) resulted in 3.1% higher weight gain and 5.9% higher FCR. Additional testing is under way, as are initial sales. NeoMAG™ is a first of its kind insect derived feed additive, on trajectory to improve livestock yields in a sustainable fashion. NeoManna is developing another BSF product, NeoPRO™, with promising anti-inflammatory activity. *In vitro* studies on immune cells show its ability to decrease inflammation, significantly reducing the excretion of key inflammatory cytokines such as TNFα and IL-6, on par with dexamethasone. In conclusion, NeoManna strives to become a leader in insect-based solutions to overcome AMR and sustain healthy, productive, and sustainable animal agriculture.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Can black soldier fly larvae replace soybean meal in poultry diets? Comparison of broiler vs indigenous chickens

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Black soldier fly (*Hermetia illucens*) larvae (BSFL) gain attention as an alternative feed ingredient with higher protein content, suggesting the possibility of replacing soybean meal (SBM) in poultry diets. The research on BSFL in poultry diets has resulted in a wide range of inclusion levels of BSFL being recommended due to confounding factors such as processing of BSFL (full fat vs defatted, live vs dried meal) and the bird type being overlooked in previous studies. Compared to commercial broilers, indigenous poultry, in particular, naked neck chickens (NN), depict a greater degree of heat tolerance mainly through genetic variation in feather development. It, therefore, can be hypothesized that two types of chickens, Cobb-500 broilers and NN, have different capacities to utilize BSFL in their diets. The influence of three levels of live BSFL inclusion (0%, 15% and 30% of SBM) was tested for Cobb-500 broilers and NN chickens in a 2 × 3 factorial arrangement. Isoenergetic and isonitrogenous experimental diets were formulated to meet the nutrient requirements by Cobb-500 for the grower (14-21 d) and finisher phases (22-35 d). A total of 180 7d-old birds (90 broilers and 90 NN) were randomly distributed among 30 cages (five replicates/treatment, six birds/replicate). Birds were fed experimental diets from 7 to 35 d and first 7 days were considered as the acclimatization period. Based on growth performance (14 -35 d), the responses of body weight gain (BWG), and feed conversion ratio (FCR) to bird type interacted with BSFL inclusion level ($P < 0.05$). Both BWG and FCR of Cobb-500 were improved in 0% and 15% BSFL compared to 30% BSFL in the diet. However, BWG of NN was not influenced by the increasing dietary inclusion of BSFL. Compared to the control diet (100% SBM), BSFL inclusion at 15 and 30% of SBM impaired the FCR of NN. Based on BWG, BSFL could replace up to 15 and 30% of SBM for broilers and NN, respectively, without comprising the BWG.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Can insects captivate pets? Effects of insect-based ingredients on pet food palatability

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The forefront of pet food innovation has seen a notable surge in the adoption of insect-based ingredients over the last three years. Given the inherent challenge of building trust and loyalty with pet owners in a crowded brand messaging space, the most compelling strategy lies in captivating pets' genuine interest and continuously creating delightful meal experiences for them. Our research explores palatability effects of insect ingredients in two scenarios: Firstly in diets where insect protein provides a significant nutritional contribution (e.g. inclusions of 10% and above and where the insect ingredient is often part of the product branding). And secondly diets where the insect ingredient has a primarily functional purpose at lower inclusions (e.g. in the range of 0.5-4%) together with conventional nutrient sources (where the insect ingredient may not play a role in the product branding). Our research covered treat, dry kibble and wet food formats and included both in-house formulated diets as well as conventional commercial diets. Trials were performed either as single-bowl acceptance tests or as two-bowl preference tests with 10-50 dogs per trial. In a single-bowl wet pet food acceptance test with a 50% insect-inclusion, 84% of dogs finished the food in one sitting (n = 52). In another trial, an insect-based coating was applied at 3% to dry kibbles vs a lamb based commercial kibble. First sniff and first finished metrics were on par and a 60% preference on first bite was recorded for the insect-coated kibble. The results provide evidence that insect ingredients can invoke favourable sensory responses from cats and dogs compared with conventional meat-based ingredients. Broadly, our results add to the existing body of knowledge towards the feasibility of insect-based diets. Specifically, they demonstrate the opportunity for functional insect ingredients to serve as a novel and sustainable source of palatability for the pet food industry, paving a pathway for adoption in a wider range of products.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Industrial scale studies on feeding and post-feeding effects of *Hermetia illucens* live larvae on egg quality

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To date, an array of research data elucidated the effects of feeding layer hens with products from Black soldier fly (*Hermetia illucens*; BSF) larvae, providing core understanding in this research area. Data from industrial studies, however, is limited. To address this, the study set an experiment in one of the largest egg farms in Bulgaria with a total of 2,800 hens – floor rearing system. The control and the experimental groups were separated by mesh, and in the experimental group in-house made device was installed to facilitate hens feeding with live larvae. Both groups were fed with the same feed, the experimental group however, received 20 g/hen/day live larvae (12-16 days old) for a period of 8 weeks. The aim of the study was to assess the impact of the additional feeding with BSF at the end of the feeding period as well as 3 weeks after. Results suggest that although egg weight did not change in both groups, egg yolk responded to the BSF feeding with increased weight (16.68 g; $P = 0.000$) compared to the control (15.91 g). BSF-fed hens, however, produced eggs with paler egg yolk colour (7.69 units; $P = 0.000$) compared to the control (8.37 units). Importantly, following assessment at 3 weeks post-feeding the egg yolk weight continued following the same trend as above, but no difference in the colour of the egg yolks from the control and experimental groups were observed. Importantly, the thickness of the egg shell responded positively to BSF feeding (con-0.343 mm; exp-0.365 mm; $P = 0.000$) and this trend persisted in the post-feeding period. Egg yolk cholesterol levels were not affected by the intake of BSF and similar values for the control were noted (con-11.25; exp-11.05). The amino acid and fatty acid levels were also investigated to show the degree of the impact of BSF. Overall, results from the present study validated specific trends on the impact of BSF as feed for hens (industrial scale), and importantly, provided interesting spatial patterns on the egg parameters at the post-feeding period to suggest the possibility of developing economically viable models of feeding hens with BSF products.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Effects of BSF inclusion on koi fish on growth, coloration, immune gene expression, and economic return

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The present study aimed to investigate the effects of black soldier fly substitution meal and supplementation with red dragon fruit peel on skin coloration, physiological performance, and immune gene expression in koi fish. Two separate feeding trials were performed in this study, focusing on 1-month-old koi. In the first feeding trial, 150 juvenile koi fish were tested with five diets developed with different concentrations of BSF in fishmeal substitution, i.e. basal diet with 100% fish meal (BD), 5% BSF, 10% BSF, 15% BSF, including a commercial diet for a duration of 45 days. Physiological parameters such as growth, survival, and food conversion rate (FCR) and coloration were determined for the optimal BSF formula. Koi liver samples collected from the first trial were then subjected to mRNA library sequencing and immune gene analyses. Weight gain, length gain, and FCR were significantly improved in the BSF group. Results from mRNA library sequencing suggested there is a slight improvement in immune genes, lower levels of growth hormone production, and decreased oxidative stress in the BSF group. Colorimetric and imaging analyses revealed that koi fish fed the BSF 15 diet exhibited moderate body coloration enhancement. In the second trial, the optimal BSF diet was supplemented with RDFP, same procedures were then performed as aforementioned, except for immune gene expression analyses. A significant improvement in weight gain, SGR, FCR, and redness was observed in the koi fish fed with BSF+RDFP diet compared with the BD and CD diets. The economic evaluation also highlighted that the profit index of BSF+RDFP was significantly higher than that of CD. Our data suggest that BSF and RDFP could be promising feed formulations for enhancing the growth, immunity, coloration, and profitability of koi. These findings have practical implications for the ornamental fish industry, as they offer a sustainable and cost-effective alternative to traditional fishmeal diets.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly meal inclusion in seabream and salmon diets: effects on growth, fish quality and health performances

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For several years, the aquaculture industry has been searching for alternative sources of proteins to respond to the growth of the sector and the challenges of sustainable development. Various studies were performed during the NextGenProteins project, assessing the zootechnical performances of four species when fed with three emerging raw materials among which were insects. Two dose response trials and two field studies were conducted with seabream and salmon. Both dose response trials were useful to determine the best inclusion level for the field studies. The dose response trials for salmon (from 81 g to 180 g) took place in Iceland with feeds designed to replace fishmeal (FM) and soy protein concentrate with insect meal (IM). A field study took place in Scotland with feeds developed to achieve 10% inclusion of IM. During this five month trial, salmon grew from 2.0 kg to 3.5 kg. The on-field trial on salmon conducted to similar weight gain and feed conversion ratio between IM diet and control. The general fish welfare was also good for both diets. The dose response trial for seabreams took place in Italy. The feed was designed to replace FM from the control with three inclusion levels of IM. The field study took place in Croatia with feeds developed to achieve 10% of IM inclusion. On seabream results allowed us to conclude that IM can replace FM without compromising growth and feed utilization. No differences were found in fillet's technological quality, oxidative state, and sensory evaluation. Therefore, IM determined a positive gut microbiota reconfiguration promoting beneficial taxa such as Paenibacillus and Bacillus potentially able to support chitin digestion and local immunity. Globally, the outcomes of NextGenProteins project allowed us to conclude that IM has a great potential to be used by the fish industry.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly meal and dehydrated larvae inclusion in shrimp diets: effects on growth and health performances

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For several years, feed manufacturers are searching for new sources of proteins to respond to the growth of the sector and the challenges of sustainable development. At the same time, more than 30% of the world agricultural production is wasted. Black soldier flies (*Hermetia illucens*, BSF) make it possible to valorize food waste and represent an adapted resource to feed shrimps because it is natural, safe, sustainably produced and has a good nutritional quality. The objective of two studies carried out in Belgium in 2020 and 2021 and one study carried out in Ecuador in 2023 was to evaluate the performances of shrimps (*Penaeus vannamei*) when fishmeal (FM) is replaced by BSF meal in comparison to a conventional feed (CTRL). In the trials, a BSF meal produced in France or in Ecuador is incorporated into feeds at different inclusion rates as replacement material for FM (from 33 to 100% of replacement). These feeds have been used to feed shrimps from 0.24 g to 10.3 g. In the first trial, the optimal inclusion level was 12.7% (66% of FM replacement), the specific growth rate (SGR) was significantly better than the CTRL for this group of shrimps (*P*-value = 0.042). An increase in average final weight up till 16.8% for this group could be observed after 28 days. At the end of a complete culture period this would result in a substantial increase in productivity. In the second trial all dietary treatment containing BSF meal showed improved performances (weight gain, SGR, feed conversion ratio and survival rate) compared to CTRL even if not statically significant. In the third trial, the best result was for the shrimps feed with dehydrated grinded larvae replacing of 50% of FM (10.6 g; not significant). Our experience and the literature suggest a positive impact of BSF meal on growth performance in shrimp. These trials ended with bacterial challenges which showed promising results that will be presented.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Use of insects in companion animal nutrition: processing and functional applications

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The use of insects as a protein source for animal feed, including pet food, has gained attention due to their high nutritional value and potential to reduce the environmental footprint of animal agriculture. Using insect protein, such as black soldier fly (*Hermetia illucens*) larvae meal (BSFLM), presents a promising alternative. Current research in our laboratory demonstrated that BSFLM has similar or superior protein quality than common protein ingredients in pet foods; whole powder egg and chicken meal. Methionine + cysteine and phenylalanine + tyrosine were often the first limiting amino acids, according to DIAAS-like score using the cecectomized rooster model. In addition, BSFLM can be easily incorporated in extruded diets at inclusion levels up to 30 % without negatively affecting processing conditions, those included preconditioner steam pressure, extruder shaft speed and motor load, as well as drying temperature and retention time. Inclusion of BSFLM increased kibble diameter and length, as well as bulk density, but these changes did not negatively affect average daily food intake. Dogs fed diets containing BSFLM as a partial or main protein source in extruded diets remained healthy, did not show any adverse food reaction, and had comparable apparent total tract digestibility for dry matter, crude protein, and fat to the control diet (chicken by-product meal as the primary ingredient protein). Overall, our results indicate that BSFLM is a safe, nutritional adequate, and suitable protein source in canine nutrition.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Effects of feeding live larvae (BSFL) on growth performance and fatty acid profile in muscle and liver of broilers

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The fatty acid (FA) composition of broiler meat basically depends on the dietary FA profile. Black soldier fly larvae (BSFL) are rich in lauric acid (LA, C12:0), which has claimed health benefits for both animals and humans. This study investigated the effects of feeding live BSFL on growth performance and the FA profile in muscle and liver of broiler chickens. A total of 36 one-d-old male broiler chicks (Ross 308) were allocated equally (3 pens/treatment) to two dietary treatments: CON and BSFL. All birds were fed ad libitum with a commercial broiler diet (basal diet), for 42 days. Birds in BSFL group were fed live BSFL in addition to the basal diet: d1-21, 5%; d21-42, 10%; of expected daily feed intake (DM basis). Growth performance data were collected weekly. On day 42, samples of pectoralis major muscle and liver were collected to determine the FA profile using gas chromatography. Broiler-fed BSFL had a higher BW (+6.5%, d42; $P < 0.05$) and higher carcass weight (+6.7%, d42; $P < 0.05$) but no difference in dressing percentage compared to CON. BSFL-fed birds had a lower ADFI of basal diet (-7%, d1-42; $P < 0.05$), while the ADFI (DM) of basal diet with larvae did not differ ($P > 0.05$). The FCR of basal diet was significantly lower in BSFL ($P < 0.05$). FCR of basal diet with larvae showed a tendency for improvement ($P < 0.1$). The whole-body composition analysis showed a trend of increasing protein content but no differences in crude fat and crude ash content of BSFL-fed birds. The BSFL feeding changed the FA profile in the intramuscular fat (IMF) of the birds. Saturated fatty acids (SFA) in IMF were significantly increased. The increased SFA are primarily due to the increase in C12:0, which was found to be 5% of total FAs. In the liver, there was an increase in C14:0 and C18:0 compared to CON, while LA was at an undetectable level. In conclusion, feeding 5-10% live BSFL increased growth performance of birds. BSFL, rich in LA, also accumulated in the IMF of breast muscle of broilers.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

In vivo digestibility of the whole meal and defatted black soldier fly meal in Nile tilapia fries diet

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A current research priority in animal nutrition is the active search for sustainable feedstuffs to reduce the dependence on FM (fish meal) or FO (fish oil). Their quality is particularly of concern in the tilapia hatchery sector, as it is heavily relied upon during the sex-reversal process of the fry. Because of its high palatability it maximises the hormone intake during the sex-reversal process in hatcheries, and helps to achieve a near 100% all male sex-reversal. The possibility to produce a FM-free feed based on a multi-ingredient formulation requires the determination of apparent digestibility coefficients (ADC's) of the different feedstuffs. Since the volume of insect meal are still too low to supply the aquaculture sector, a targeted use should be made, in this aim we investigated the ADC's of black soldier fly meals (defatted and whole) as a high-quality feed source for tilapia fry during their sex-reversal process. To calculate the ADC's the method using a basal diet containing Yttrium oxide as an inorganic marker was used. Both meals, were proven to be good feed ingredient even showing in our case, better digestibility values for proteins than fishmeal (>99%) and could be used as a substitute of fish meal in their sex-reversal diet, as part of a fully formulated feed.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly larvae fat metabolizable energy value implemented in broiler chicken diets

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The present study aimed to investigate the apparent metabolizable energy (AME) and apparent metabolizable energy corrected to zero nitrogen balance (AMEn) levels of H. illucens (BSF) larvae fat for broiler chickens of various ages. 400 1-day-old male Ross 308 birds were randomly assigned to four dietary groups (10 replicate pens per treatment; 10 birds per pen). The following treatments were applied: HI0 – basal diet without dietary fat inclusion, HI03 – basal diet enriched with 30 g/kg BSFlarvae fat, HI06 – basal diet enriched with 60 g/kg BSF larvae fat, and HI09 – basal diet enriched with 90 g/kg BSF larvae fat. Broilers had ad libitum access to mash form feed and water. Excreta samples were collected on d 14, d 28, and d 35. The simple linear regression method was used to establish the AME and AMEn values of BSF larvae fats. The results show that the AME and AMEn values of BSF larvae fat for broiler chickens are 9 049 kcal/kg and 9 019 kcal/kg, respectively. Furthermore, because the birds' age significantly affected the AME and AMEn levels, the implementation of BSF larvae fat to broiler diets should be considered in each nutritional period using the recommended regression model AME = 2 559.758 + 62.989 × fat inclusion (%) + 7.405 × day of age and AMEn = 2 543.2663 + 62.8649 × fat inclusion (%) + 7.3777 × day of age. The data highlighted that the BSF larvae fat metabolizable energy level is similar to soybean oil. However, the authors do not recommend using the regression equations mentioned above to calculate the energy level of BSF larvae fat for young birds, i.e. before 14 d of age. 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 AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Hermetia illucens larva fat affects broiler chicken breast meat quality

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This study evaluated the dose-dependent effect of black soldier fly (BFL) larvae fat inclusion in broiler chicken diets on breast meat quality. Four hundred 1-day-old birds were assigned to the following four treatments (10 replicates, 10 birds each): HI0, a basal diet without dietary fat inclusion, and HI03, HI06, and HI09, basal diets enriched with 30 g/kg, 60 g/kg, and 90 g/kg of BSF larvae fat, respectively. Principal component analysis showed a noticeable differentiation between the selected plant, animal, and insect-origin dietary fats. The BSF fat exhibits a strong relationship with saturated fatty acids (SFAs), resulting in a high concentration of C12:0 and C14:0. The fatty acid (FA) profile in breast muscle obtained from broilers fed diets with increasing insect fat inclusion showed a significant linear effect in terms of C12:0, C15:0, C18:2, C18:3n6, and total FAs. The proportion of dietary insect fat had a quadratic effect on meat colour. The water-holding capacity indices have stayed consistent with the meat colour changes. Throughout the experiment, favourable growth performance results were noticed in HI06. The present study confirmed that BSF larvae fat negatively affects the n3 level in meat. However, the physicochemical indices related to consumer acceptance were not altered to negatively limit their final decision, even when a relatively high inclusion of insect fat was used. 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 AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

The effect of dietary fats and insect oil inclusion in broiler diets on the gastrointestinal tract microbiota

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The study aimed to determine the effect of selected dietary fats administration in broiler chicken diets on the gastrointestinal tract microecosystem. In total, 800 one-day-old chicks (Ross 308) were randomly assigned to 8 experimental groups (10 replications, 10 birds each). Chickens were fed ad libitum from day 1 to day 35. The design of the experiment was set up as follows: HI – fat derived from *H. illucens* larvae; SO – soybean oil; RO – rapeseed oil; PO - palm oil; PKFD – palm kernel fatty acid distillate; PF – poultry fat; PL – lard; BT - beef tallow. At the end (35 d), the digestive tract contents, i.e. from the crop, jejunum, and ceca, were collected for further microbiological analyses. In the crop content, an increase in the total number of microorganisms (DAPI) in SO, RO, PO, PL, and BT was noted compared to HI. In addition, increased proliferation of *Clostridium perfringens* was noticed, and Lactobacillus/Enterococcus spp. in BF and C. coccoides/Eubacterium rectale cluster in PKFD. Regarding the size of the other analyzed microbiota populations, the Bacteroides-Prevotella cluster, *C. leptum* subgroup, and Enterobacteriaceae, no significant differences between treatments were found. In the jejunum, a decrease in DAPI in PKFD was noted in relation to HI. Both vegetable oils (PO and PKFD) and animal fats (PF and BT) reduced the number of *C. perfringens*. Moreover, in the SO, PO, and BT groups, there was an increase in lactic acid fermentation bacteria. In the case of the cecal microecosystem, an increase in the proliferation of the total number of bacteria was observed in the PF and PL groups compared to the HI group. The use of *H. illucens* larvae fat does not exhibit a negative impact on the chicken gut microbiota, and its effect is comparable to that of commonly used soybean oil. 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 AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Effects of selected dietary fat inclusion in broiler diets on growth performance, digestibility, and meat quality

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This study investigated the effects of the administration of selected dietary fats in broiler chicken diets on growth performance, selected organ and gastrointestinal tract morphometrical measurements, nutrient digestibility, and breast meat quality. A total of 800 one-day-old male (Ross 308) chicks were randomly divided into eight dietary groups, with 10 replication pens containing 10 birds each. The dietary treatments included selected dietary fat sources: *Hermetia illucens* (HI) fat, palm kernel fat distillers (PKFD), soybean oil (SO), poultry fat (PF), palm oil (PO), rapeseed oil (RO), beef tallow (BT) and pig lard (PL). The experiment lasted 35 days. The provided dietary fats of plant origin had higher concentrations of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), those of animal origin were higher in saturated fatty acids (SFAs), and HI fat was characterized by high lauric and palmitic acid contents, as well as SFAs. The growth performance parameters, organ measurements (weight and length), nutrient digestibility coefficients, and apparent metabolizable energy corrected to zero nitrogen balance (AMEN) were not affected ($P > 0.05$), except for FCR ($P < 0.001$) and the relative jejunum weight ($P = 0.009$). The fatty acid profile of breast meat varied across dietary fats ($P < 0.001$), impacting the PUFA/SFA ratio ($P < 0.001$). The study confirmed that HI fat can replace commonly used dietary fats in broiler chicken diets without adverse effects on growth performance, nutrient digestibility, and meat quality. 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 AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Insects as bioconversion drivers of the alternative protein-based animal feed value chains

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The dependency of European agri-food systems on imports of the main protein sources renders them unstable and unprotected from major disruptions of the different channels in the supply chain, as it has been proven during the pandemic crisis. Most agricultural farming systems produce a huge amount of livestock and crop residues, as well as a variety of side-streams. As agri-food side-streams and by-products represent a threatening biomass for the public healthy and the environment, novel approaches to waste management must be developed, and insects have been already proposed as efficient agents for biorefining waste, producing biomass that can be used for commercial feed products. Insect production has been reported as having a lower environmental impact than other sources of animal protein, such as beef, pork, or chicken meats (e.g. lower GHG emissions, the need of much lower areas of land for their rearing, higher efficiency in the utilization of energy, much lower inputs of feed and water). Nevertheless, issues can also be pointed out, such as those related to the costs associated with heating the insect production sites in temperate climate countries, control of pests and diseases, or maintenance of hygiene, which have been shown to considerably reduce the attractiveness of food insect farming. The aim of this presentation, which is building on the activities and initial outcomes from several funded projects (CIPROMED, Advagromed) is to showcase several potential best-case scenarios for sustainable valorisation of insects within the animal feed value chain. The utilisation of insects in commercial formulas for animal feed represents a possible way to turn animal diets into more sustainable systems, since insects can constitute a better source of protein when compared with the present commonly used sources. By integrating insects in a systemic approach, together with side-streams, such as agri-industrial residues and by-products, but also blending with other, cost-effectively generated protein meals in feed, the aim is to increase the circular economy attributes, along with a wide range of Life-Cycle-proved economically and environmentally sustainable concentration, modification and formulation techniques.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Novel protein sources for animal feeding: the CIPROMED project

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CIPROMED project aims at upcycle and valorize agro-industrial side-streams for insect rearing and microalgae cultivation, thereby applying the Circular Economy principle. The novel protein sources obtained are used as ingredients in innovative feed for fish and poultry. In the work package 5, animal trials are planned to test an insect meal (*Hermetia illucens*) and a blend of insect and microalgae (*Chlorella vulgaris*) meal for fish (rainbow trout, European sea bass, and seabream) and broiler chickens. First, digestibility of the novel protein sources is performed and results used to formulate diets for performance trials where increasing levels of the novel proteins are tested. To assess the impact of bioactive compounds contained into the novel proteins, challenge tests are performed. General and gut health (histology and microbiota) is analyzed in all considered animal species. Moreover, physical and chemical product quality is assessed. In addition, trials are carried out on juvenile rainbow trout and sea bream, to evaluate two insect species that are not among the ones authorized in the EU: the Mediterranean fruit fly (*Ceratitis capitate*) and the Oriental fruit fly (*Bactrocera dorsalis*). These species could be used in other non-EU countries and therefore the results could be relevant for the aquaculture sector at a global level. (Supported by the EU-PRIMA program project CIPROMED).

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Farmed black soldier fly for poultry

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The aims are to offer novel means to improve feed protein self-sufficiency in poultry farms as well as to improve security of supply and resilience of farming in changing world. The need for alternative animal feed protein produced in farm or near-by area is currently highest in organic production due to increased fish meal prices and limited availability of organic soy. To this end we produce an operations model for black soldier fly (*Hermetia illucens*) maggot protein production in farm scale. The model will be scalable and generally applicable. The operations model will be tested, and the results published and put available for everyone. An information on methods and equipment needed to produce *Hermetia* protein and calculation tools for farm-specific profitability calculations to estimate the economical sustainability will be included in the model. Once a clear generalizable model is available, starting and scaling the production up will be easier. The calculation tools will help to assess the conditions under which maggot protein production would be economically viable under changing situations and cost factors. At present we are testing feasibility of cereal grain sorting side streams as *Hermetia* feeds. In Finnish farms c.a. 15% of the grains go to sorting side streams. In pilot tests a *Hermetia* feed porridge from 4.5 kg of sorting side streams (barley 2 kg, wheat 1 kg, pea 0.5 kg, rapeseed 1 kg) + 5.5 kg water, resulted to c.a. 3.3 kg of living maggot and to 2.1-2.8 kg of frass in 12 days at ambient temperature of 13-15 °C and RH 40%. These preliminary results suggest that these sorting side streams are feasible for mass production of *Hermetia*. The grain crushing and resulting particle size as well as the structure of the porridge are of critical importance for the optimal maggot growth. We are testing different ways to crush the grains and compose the porridge and will publish the results in future. This project is funded by The European Innovation Partnerships – Project (EIP), where the goal is to create new approach to research and innovation.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly products inclusion in broiler diets: effects on animal performance, meat quality, health, and welfare

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Black soldier fly (BSF) can valorize various food waste and can thus be produced all over the world. It represents a particularly adapted resource to feed broilers because of its nutritional qualities and its potential to be used as an environmental enrichment to improve animal welfare. The objective of PINHS project ("Poultry fed with Insects: Nutrition, Health & Sustainability") was to evaluate the effects of three products derived from BSF larvae to feed fast-growing broilers: a BSF defatted meal and whole live or dehydrated larvae. The nutritional value of the products was assessed in a digestibility trial. Results showed that despite a lower digestibility of nutrients in BSF meal (compared to whole larvae), this product is richer in digestible amino acids, confirming its potential to replace soybean meal. The second trial evaluated the effects of whole BSF larvae as an enrichment material (4% of dry matter intake) vs environmental enrichment (NE) vs the use of wheat grains (W). Results showed that the activity of birds receiving larvae was higher compared to NE and W treatments, but the effects of dose and number of daily distributions should be further investigated. The last trial evaluated the performance of broilers fed with pelleted feed containing 10% of BSF meal or with a deconcentrated feed completed by the provision of whole larvae (15% of dry matter intake). Results showed that the use of BSF meal or live larvae led to similar animal performance or carcass and meat quality when compared to the control. On the opposite, the provision of a large quantity of dehydrated larvae impaired growth and consequently meat yield, probably because the gut was cluttered affecting digestive transit time. The use of live larvae increased lipid peroxidation and haptoglobin content in blood but also the antioxidant status compared to birds fed without BSF. In general, the outcomes of the PINHS project allowed us to conclude that insects' products have a great potential to be used by the poultry industry in Europe.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Different uses of *Hermetia illucens* in the post-weaning piglets

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Black soldier fly larvae (BSFL, *Hermetia illucens*) are an excellent candidate as a source of protein and lipids and could be an interesting element of environmental enrichment aimed at reducing re-directed exploratory behaviours (e.g. aggression, tail biting) in weaned piglets. The aim of this study was to evaluate growth parameters and behaviour of piglets receiving larval meal (LM) and/or live larvae (LL) in addition to environmental enrichment. A total of 96 piglets, 28 days old, were individually weighed and allocated to different treatments. The trial lasted 39 days and there were 4 experimental groups, each consisting of 4 replicates of 6 piglets each: a control group (C), a control group with live larvae (CLL), a group receiving larvae meal (LM), and a last group receiving both live larvae and larvae meal (LMLL). BSF larvae meal was included at 6%, replacing an equivalent amount of soybean meal; all 4 groups had standard environmental enrichment (iron chain and softwood); the two LL groups received live larvae from a special dispenser at increasing levels during the trial, up to a maximum of 600 gr/pen (100 gr/piglet)/day. At the end of the trial, piglets were individually weighed, average daily gain (ADG) was calculated and feed conversion ratio (FCR) was calculated from feed intake. Video recordings were made to obtain behavioural data. Evaluation of the number and duration of events is still ongoing. The administration of larval meal and live larvae to piglets in the post-weaning period has no significant effect ($P > 0.05$) on growth performance (live weight, average daily gain, feed conversion ratio). Preliminary behavioural results show that animals receiving live larvae spent approximately 15% of their time interacting with the larvae. As a result, interactions between piglets and with traditional environmental enrichment (wood and chain) were significantly reduced. These differences occurred regardless of whether BSF meal was present or not and indicate a promising effect of LL on piglet behaviour.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly meal inclusion in broiler and turkey diets: effects on growth performances, meat quality and health

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For several years, feed manufacturers have been searching for new protein sources to respond to the growth of the sector and to the challenges of sustainable development. Several studies were carried out during the NextGenProteins project (EU Horizon 2020 – 862704) with the aim of assessing the growth performances of poultry and fish when fed emerging protein sources, including insects. For poultry, experimental dose-response trials were conducted to evaluate the effects of partial substitution of soybean meal with black soldier fly meal (BSFM) on traits related to productivity, welfare, metabolism, and product quality. Then, in-field validation trials were carried out to confirm the outcomes of the dose-response studies. The dose-response trial showed that the inclusion of 9% BSFM leads to similar feed conversion efficiency compared to a soybean-based diet, while 18% worsened productive performance and body weight uniformity. At both inclusion levels, no adverse effects on welfare and meat quality traits were observed. For the in-field trials, feeds containing 9 and 5% BSFM were provided to broilers (growing and finishing) and to female turkeys (from 64 to 105 days), respectively. Overall, BSFM-fed broilers achieved similar performances than those receiving a commercial diet. Turkeys fed diets with 5% BSFM presented better performance than control birds (body weight: 10,173 vs 10,057 g/bird; feed conversion ratio: 2.127 vs 2.141; respectively, $P < 0.05$). Gut microbiota of turkeys was similar between treatments while an increase in butyrate level in the ceca has been observed in the BSFM group, which could be linked to gut health conditions. The meat quality parameters did not show any difference between treatments except for a slight reduction of yellowness. Overall, the outcomes of the NextGenProteins project allowed us to conclude that BSFM has great potential to be used in poultry feeding.

INSECTS AS FEED INGREDIENTS, IN DIET FORMULATIONS, FOR GROWTH AND HEALTH OUTCOMES

Black soldier fly larvae (BSFL) and frass as protein supplements for beef steers consuming low-quality forage

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Increased global meat consumption has led to an increase in livestock production. While the demand for output increases, so has the need for alternative protein sources in feed to minimize the environmental impact of livestock production. Insect protein is a potential alternative to conventional sources of protein (e.g. soybean meal or cottonseed meal) for cattle consuming low-quality forage (LQF). The objective of this experiment was to evaluate the use of black soldier fly larvae meal (BSFLM) and BSFL frass and larval sheddings (FRS) as novel supplemental proteins for beef steers consuming low-quality forage. Eight ruminally fistulated steers (240.2 ± 22.5 kg of BW) were used in replicated 4 × 4 Latin squares. One of four treatments were provided to each steer per period: a control (CON) with no supplement, cottonseed meal (CSM), partially defatted BSFL meal (BSFLM), or BSFL frass and larval sheddings (FRS). Four 16-day periods were conducted with an 8-d adaptation to treatments, 7-d measurement of intake and digestion, and 1-d collection of ruminal fermentation and microbial samples. Dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) were determined for forage, supplement, ort, and fecal samples. Protein supplementation of CSM, BSFLM, and FRS increased forage organic matter (OM) intake ($P < 0.01$) relative to CON with no significant differences between CSM and FRS ($P = 0.84$) or BSFLM and FRS ($P = 0.13$) and with a trend for a difference between CSM and BSFLM ($P = 0.08$). Provision of BSFLM and FRS increased total digestible OM intake (TDOMI) relative to CON ($P < 0.01$), from 2.33 kg/d for CON to 3.07 kg/d for BSFLM and 3.05 kg/d for FRS. Overall, our results indicate that BSFLM and FRS can be incorporated as a protein source for beef steers consuming low-quality forage.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Black soldier fly frass with antagonistic properties against phytopathogenic fungi *Foc* TR4 and *Ganoderma boninense*

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Black soldier flies (BSF, *Hermetia illucens*) are detritivores that are increasingly used for organic waste recycling and sustainable animal feed production. BSF production facilities generate large quantities of frass which can be used as an organic fertiliser and soil amendment. Here we present evidence demonstrating *in vitro* and *in vivo* assays of two globally significant fungal plant pathogens by BSF frass preparations. BSF frass preparations inhibited *Foc* TR4 by up to 90% and *Ganoderma boninense* by up to 82% in multiple antifungal assays including disk diffusion, sample-amended medium and dual layer agar methods. Biochemical assays of the frass preparations demonstrated the presence of fungal inhibitory compounds, enzymes and microorganisms including siderophores, chitinases-, protease- and lipase-producing bacteria. For *in vitro Foc* TR4 bioassay, the frass exhibited an inhibitory percentage ranging from 47-90%. Findings from greenhouse studies demonstrated that plants treated with the frass resulted in a Disease Severity Index as low as 4.17% vs 100% in the untreated control, and displayed up to 47.66% efficiency in reducing disease incidence. Treatments at higher temperature and lower humidity level also demonstrated a significant reduction in disease progression. In *in vitro Ganoderma boninense* antifungal assay, the frass exhibited an inhibitory percentage up to 82%. The greenhouse test conducted is a pot experiment using 2-months old tissue cultured Cavendish banana plantlets (variety susceptible to TR4) consisting of 23 groups of treatments with 10 plants for each treatment. This study presents evidence that BSF frass is not only an organic fertiliser but also has disease suppressive traits that could be used against globally significant plant pathogens.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Reassessing composting conventions: insights from industrial black soldier fly frass composting

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Research and guidance on large-scale composting systems consistently recommend initial Carbon-to-Nitrogen (C:N) ratios between 25:1 and 30:1 across organic inputs. This range is thought to foster efficient decomposition and avoid significant nitrogen loss through ammonia volatilization. However, this study revisits this assumption in the case of composting black soldier fly larvae (BSFL) frass. We explored the distinct nitrogen dynamics associated with the BSFL feeding phase and composting of the subsequent frass. We investigated the role of composting in an industrial windrow composting system. Windrows with 20 metric tons of unamended frass were maintained at an average moisture level of 35% and turned every two days for 35 days. Our investigation reveals composting unamended frass with low initial C:N ratios between 9-12 resulted in a mature compost with low nitrogen losses and without the processing and quality issues experienced using equivalent C:N ratios in non-frass composting systems. Data after composting frass across 6 batches showed an average of 4.3% nitrogen and 10.3 C:N ratio indicating no significant loss in nutrition value of frass when composted. When utilized in composting, frass exhibits ease of composting and stable nitrogen content, primarily attributed to the nitrogen immobilization and ammonia volatilization that happens during larvae-rearing. Microbiome composition, nutrient cycling mechanisms, and carbon availability in larvae feed may all contribute to this phenomenon. Further research is needed on the initial C:N ratio of the larvae feed and the impact this ratio has on nitrogen dynamics compared to the ratio in frass. Given our findings, additional high-carbon amendments may or may not be needed depending on the C:N ratio in the starting larvae feed. This has major implications for industrial BSFL companies who are put off composting frass due to the assumed need to purchase high-carbon additives. The findings underscore the need to re-evaluate the assumptions of industrial composting in the context of the unique production features of BSFL frass. These strategies can increase frass quality, positioning it as a premium organic fertilizer.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Diversification of insect frass fertilizer to suit production requirements and economic conditions

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Insect frass fertilizer (IFF) is a promising technology globally, but its application as a multipurpose, cost-effective, and regenerative intervention to improve soil health, crop productivity has not been fully exploited. Compared to other commercial organic fertilizers that are available in powdered, liquid and pelletized forms, IFF is currently available in powdered form only. This study aimed to develop diversified black soldier fly frass fertilizer (BSFFF) products to suit different production requirements, accelerate wider product commercialization, and fertilizer and food self-sufficiency. A three-step BSF-mediated waste bioconversion technology was utilised to develop solid, liquid, granulated, and chitin-fortified BSFFF products. The BSFFF produced were analysed for macronutrients, secondary nutrients and micronutrients, and agronomic effectiveness of different BSFFF formulations was assessed using beet root crop. Results revealed all BSFFF products had higher concentrations of all the essential nutrients required for optimal crop growth compared to the commercially available conventional fertilizers. The concentrations of nitrogen (1.5-8.5 folds), phosphorus (1.1-11.3 folds) and potassium (1.9-3.9 folds) in liquid BSFFF were higher compared to those of the commercial liquid fertilizer. Likewise, the solid BSFFF had higher concentrations of nitrogen (1.3-4.4 folds), phosphorus (2-2.6 folds) and potassium (1.1-3.7) compared to conventional solid fertilizers. Fortification of BSFFF with BSF pupal exuviae significantly improved the concentrations of nitrogen (6-54%) and phosphorus (8%) compared to unamended BSFFF. Agronomic studies showed that all BSFFF significantly increased beetroot growth, yield and net income compared to unfertilized soil. Our findings show the superior quality and high agronomic efficacy of all BSFFF products developed. It is anticipated that diversification of IFF products will increase their utility and adoption for improved food and nutrition security through circular economy.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Physico-chemical and functional properties of insect chitosan: comparison with crab and shrimp chitosan

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This study was conducted to determine and compare physico-chemical and functional properties of chitosan purified from black soldier fly (*Hermetia illucens*; BSF), blue swimmer crab (*Portunus pelagicus*) and white leg shrimp (*Litopenaeus vannamei*) shells. Chitosan was purified from shells using a method consisting of pre-treatment, demineralization, deproteinization, pigment removal, deacetylation and purification. Purified chitosan was identified using X-Ray Diffraction (XRD) and Fourier Transform Infrared spectroscopy (FTIR), and analyzed for physico-chemical, antioxidant and antimicrobial properties. Two patterns and the peak positions of XRD of BSF chitosan was almost similar to the standard, crab and shrimp chitosan. The vibration of functional groups of BSF chitosan was similar to the standard wavelengths of standard chitosan in the FTIR as in literature, and comparable with crab and shrimp chitosan. BSF (24%) resulted lower ($P < 0.05$) chitosan yield than crab (33%) and shrimp (33%). BSF chitosan had the highest moisture and ash contents and water & fat binding capacities, and lowest whiteness index ($P < 0.05$). The degree of deacetylation was higher ($P < 0.05$) in BSF chitosan than shrimp, but lower ($P < 0.05$) than crab. According to the DPPH and FRAP assays, BSF chitosan had a higher ($P < 0.05$) antioxidant activity than crab and shrimp. BSF chitosan showed antimicrobial properties against *Candida*, *Staphylococcus*, *Pseudomonas* and *E. coli*, while the activity against *Staphylococcus* and *Pseudomonas* was higher ($P < 0.05$) than crab and shrimp. In conclusion, physico-chemical and functional properties of insect chitosan vary from crab and shrimp chitosan.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Microbiome and plant-nutrient dynamics in heat-treated insect frass: implications for soil application

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Insect farming is driven by the idea of upcycling low-value organic waste into high-quality protein and fat for animal feed production. In this process, insect farming residues (frass) constitute the main by-product and can account for more than half of the input substrate. As frass is considered an efficient organic fertiliser due to its high content of plant nutrients and beneficial microbes, it can significantly contribute to insect farm revenue. However, to maximise the added value of insect farming, a wide range of low-value organic waste is used, complicating the standardisation of frass composition and quality. Consequently, the EU has taken the first step towards standardisation by aligning frass treatment regulations with those of processed manure, requiring producers to treat frass at 70 °C for 1 h before commercialisation. However, the effects of heat treatment remain unknown. In this context, we studied the physicochemical and microbiological properties of frass from three widely farmed insect species (*Hermetia illucens*, *Tenebrio molitor*, *Gryllus assimilis*) before and after heat treatment and assessed effects of treatment on the suitability of frass for soil application. Plant nutrient content revealed that frass properties varied significantly between insect species, with concentrations of plant-available nutrients reaching 7,000 µg NH4+-N, 150 µg NO2-NO3-N, and 20 mg available P per g of total solids. Although frass plant nutrient content and microbiota composition remained largely unchanged after heat treatment, viable counts of pathogenic *E. coli* and Salmonella sp., as well as microbial activity, were significantly reduced. However, adding frass to the soil reactivated and boosted soil microbial activity, resulting in a 25-fold increase in soil respiration compared to the control soil (without frass). This suggests that there are no long-term negative effects on microorganisms. These findings not only improve our understanding of insect frass as nutrient-rich organic fertiliser, but also have implications for regulatory frameworks, highlighting its potential for promoting soil health and nutrient cycling.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Impact of BSF activities on the bioplastics in the manures

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The use of *Hermetia illucens* (Diptera, Stratiomyidae) larvae in bioconversion stands out as an innovative approach for the treatment and valorization of livestock manures. Unfortunately, microparticles from various sources of bioplastics may be present in these manures. Furthermore, the presence of microplastics (MPs) may affect the larvae's ability to efficiently degrade manure and the quality of frass. This study investigated three types of bioplastics in the experiment: (1) polylactic acid (PLA), (2) polybutylene succinate (PBS), and (83) polybutylene adipate terephthalate (PBAT). These bioplastics were finely ground using CryoMill to achieve a granulometry of 5 micrometers. About 1% of microplastics (MPs) were introduced into the rearing substrate composed of poultry and swine manure. These substrate samples spiked with MPs underwent chemical extraction and were analyzed using FTIR, TGA, and RMN techniques. Each of the three experimental trials was conducted in triplicate in plastic boxes, within a controlled laboratory environment (27 ± 2 °C and RH 60 ± 5%). Each container contained approximatively 170 of 3-day-old larvae (3 DOL) and 300 g of substrate (1/3 poultry manure and 2/3 swine manure). The larval density was maintained at 1 larva/cm^2, based on literature data and preliminary trials. The substrates were not enriched with additional substances such as water or feed. In the FTIR analysis of frass, 78% of PLA, 74.8% of PBS, and 78.2% of PBAT were respectively detected in the boxes. No trace of bioplastics was found in the control box. For PBS and PBAT, no changes in bioplastics structure were detected through various analyses (FTIR, TGA, and RMN). In the near future, the impact of BSF activities will be confirmed for higher concentrations of bioplastics and larger-sized particles and through the clear localization of bioplastics within insect tissues. Currently, the use of frass should be approached with caution.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Consolidating organic waste bioprocessing: black soldier fly frass as an effective feedstock for anaerobic digestion

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Concerns regarding the emissions of greenhouse gases from organic residues have resulted in a range of bioconversion approaches, including composting, anaerobic digestion (AD) and upcycling using black soldier fly larvae (BSFL). We hypothesized that BSFL frass could be further valorised to produce methane via AD and rationalize the 'input competition' between these two processes, as well as fertilizing properties of resulting digestate (as determined by nitrogen mineralization). Biochemical methane potential (BMP) tests were performed on a fruit/vegetable mixture representing a typical food waste (FW) profile and the frass produced this food waste. These tests allow the determination of the methane production of organic residues per volatile solid (VS) mass, expressed in $NL_{CH4}.kgVS^{-1}_{substrate}$. Similarly, anaerobic nitrogen mineralization tests (ANMP) determined the organic nitrogen (Norg) mineralization into ammonium, expressed in % ($gNH_4^+.gNorg^{-1}_{substrate}$). Methane production obtained for the frass produced from food waste bioconversion by BSFL was 299 ± 31 $NL_{CH4}.kgVS^{-1}_{frass}$. This production was 32% lower than that measured on the food waste used for the BSFL bioconversion (441 ± 44 $NL_{CH4}.kgVS^{-1}_{FW}$). The result obtained on the frass was within the range of manures and raw crop residues. Regarding resulting AD digestate characteristics, nitrogen mineralization, 14% ± 2% of organic nitrogen was mineralized, and was 50% lower than that measured for the feedstock used for the BSFL bioconversion (35% ± 3%). This low Norg mineralization is due to the fact that 29% of the frass total nitrogen was already mineralized due to larvae bioconversion and storage conditions. The nitrogen mineralization value is similar for manure with high initial ammonium concentration and low mineralization. This study is a first step to enable combined AD/BSFL bioconversion evaluation and assess optimal environmental and economical scenarios to manage food waste.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

First come, first served: impact of frass application on cabbage root fly infestation in cauliflower production systems

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The residual substrate of insect culture, frass, is the subject of a wide range of research topics. Especially in European organic agriculture there is interest in using frass as an alternative fertilizer. The composition depends on insect species, breeding protocol, processing technique etc. However, frass has more properties than just its fertilizer value. Research showed that frass affects the rhizosphere of plants and has an impact on plant development. The cultivation of cabbage crops is associated with the risk of infestation by the cabbage root fly (CRF). The larvae of this fly damage the root system, resulting in reduced crop yield or even plant loss. With the withdrawal of plant protection products, both conventional and organic farmers struggle with decreasing yields in their cabbage production systems due to the CRF. During the 2021 to 2023 growing seasons, research institutes Inagro and PCG conducted 6 field trials in cauliflowers, 3 in conventional and 3 organic farming, to assess the potential of frass as an organic and low-cost alternative technique to control the CRF. Entomopathogenic nematodes and insecticide based drench treatment before planting, were used as controls. In these trials also the use of different forms of frass were evaluated, including (pelleted) frass from black soldier fly (BSF). In each trial the density of CRF and plant losses were assessed weekly and yield determined at harvest. Results show the added value of applying insect frass in cauliflower production to trigger the plant's defence system. In particular, pelletized BSF frass applied at planting results in reduced damage by CRF larvae and consequently reduced yield loss in comparison with untreated. Over 6 field trials, the average cumulative plant loss due to CRF was 49% for untreated plants, while BSF frass application reduced this to 21% and the chemical reference Spinosad resulted in 19% plant loss. The application of insect frass in horticulture and arable crops offers clear potential. Further research for optimization of application timing and use in other crops are planned.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Insect frass as novel organic fertilizer: balancing benefits and limitations

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As the global demand for sustainable agricultural practices increases, insect-derived organic fertilisers, such as black soldier fly larvae (BSFL) frass, emerge as promising solutions. This study investigates two commercial Australian frass products using laboratory analysis and a greenhouse study investigating its effects on tomato plant growth and arbuscular mycorrhizal (AMF) colonisation. Laboratory chemical analyses showed that both BSFLs contained high concentrations of plant macro-nutrients. Total phosphorus ranged from 1.3% to 1.6% and total nitrogen from 3.6% to 3.9%, with high concentrations of available mineral nitrogen. However, 99% of the mineral nitrogen was in the form of ammonium. Total carbon concentrations were also very high around 45%, while micronutrient concentrations were more variable. A seed germination test showed that one product had a much greater negative effect on seed germination than the other. A greenhouse study was conducted in which one of the frass products was selected and applied based on its N concentration, with six treatments ranging from 0 to 250 kg N ha-1. Plants in the 150 to 250 kg N ha-1 treatments showed symptoms of ammonium toxicity in their early growth stage. AMF root colonisation was almost completely inhibited between 100 and 250 kg N ha-1. Significant growth effects of tomato biomass were already visible at the lowest application rate and reached a plateau between 150 and 250 kg N ha-1. The results show that insect frass is an effective organic fertiliser with interesting properties that can be used for sustainable food production. However, some problems were identified that could hinder its application in the field, such as high ammonium concentrations or negative effects on AMF root colonisation and seed germination. To avoid harmful ammonia levels and reduced mycorrhizal colonisation, it should be applied only at the optimum dose-yield response curve and more frequently over the growing season of a crop. To counteract nutrient imbalances, insect frass could be combined with other fertilisers or undergo a secondary composting phase.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

BSF frass antagonism against plant fungal pathogens: insights into modes of action

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Plant fungal pathogens pose significant challenges to sustainable agriculture and horticulture. Black soldier fly (BSF) frass, which is composed of chitin, larvae faeces and residual substrate, is a rich source of nutrients, beneficial microorganisms and antifungal compounds. BSF frass has been found to possess antifungal properties which can help control plant pathogens such as *Ganoderma boninense* (the causal agent of the basal stem rot disease in oil palm) and *Fusarium oxysporum* sp. Cubense TR4 (*Foc* TR4) (the causal agent of the fusarium wilt disease in bananas). The antifungal activity of frass is primarily attributed to the presence of the beneficial microbes with strong antagonism against fungi such as siderophores, chitinase, lipase and protease-producing bacteria. Across a range of biochemical assays (serial dilutions, plating on selective media and enzyme assays), we found lipase-producing bacteria at 1.22×10^6 CFU/g, protease-producing bacteria at 5×10^4 CFU/g, 48.5% of siderophores and 0.29 mg/mL of chitinase enzymes in the BSF frass. Besides its biocontrol efficiency, BSF frass could also promote plant and soil health, which is associated with the presence of the plant growth-promoting bacteria such as nitrogen-fixing and phosphate-solubilizing bacteria (5×10^3 CFU/g and 1×10^4 CFU/g, respectively). This study will provide insights into the potential of BSF frass as a natural and sustainable means of controlling plant fungal pathogens and promoting plant and soil health.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Optimisation of a cutting-edge biorefinery process for high purity chitin from black soldier fly larvae

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Black soldier fly larvae (*Hermetia illucens*) quickly and efficiently convert low-value waste streams into high-value larval biomass, containing substantial amounts of lipids (±40%), proteins (±30%), and chitin (±5%). While these larvae can be used as animal feed, extracting and isolating the aforementioned substances can significantly increase their economic value. To this end, we recently developed a cutting-edge sustainable biorefinery process. Our process uses a mixture of subcritical methanol and water to produce methyl esters, oligopeptides, and chitin from the larvae. However, as this novel process has only recently been developed, the impact of process parameters was unknown. To bridge this knowledge gap, we conducted a Design of Experiment (DoE) study, hereby focusing on highly-pure chitin given its high economic value. This approach allowed us to map the influence of key process parameters on both chitin yield and purity. It also enabled the identification of parameter interactions and quadratic effects, providing predictive insights into the behaviour of our newly developed process under varying parameter values within the defined design boundaries. Following model fitting, we were able to predict optimal process parameters for the production of chitin with the highest purity. Through the application of the DoE approach, our research has successfully optimized the process, resulting in chitin purities exceeding 80%. Moreover, the chitin fraction hardly contains any amino acids (<0.3%), implying that the covalently bound proteins have been completely removed. In conclusion, this study represents a significant leap forward in the development of a sustainable biorefinery process for isolating chitin. This process also allows for the simultaneous valorisation of both the fat and protein fractions while avoiding the use of large amounts of hydrochloric acid and sodium hydroxide.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Upcycling BSF frass as a means for improving its quality

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Waste management with black soldier fly larvae (*Hermetia illucens*, BSFL) is a worldwide established technology. The frass fertilizer obtained in waste bioconversion can be reintroduced in agriculture, however its lack of maturity hampers its use, which could be improved by further stabilization. This study aimed at improving BSFL frass quality by recirculating it within the bioconversion process, thus by providing fresh frass (obtained after one bioconversion of waste after larvae harvesting) as feed substrate for the larvae. Fresh frass was produced with post-consumer food waste (FW). Then, young BSFL were fed diets containing FW and fresh frass, with the latter being used as a replacement of FW in increasing levels (2.5% to 100%). The subsequent frass fertilizers obtained (i.e. frass resulting from the bioconversion of FW combined with fresh frass) were evaluated for nutrient content, stability and maturity, in addition to biological safety aspects. Bioconversion efficiency was reduced from 25% (0% inclusion of fresh frass) to 11% (100% inclusion). However, larval biomass produced per experimental unit remained the same regardless of inclusion level. Fresh frass inclusion as part also resulted in subsequent frass with lower organic matter content (82% $_{DM}$ in comparison to 87%$_{DM}$ in fresh frass) and higher nutrient concentration (increases up to 40%), such as P_2O_5 and K_2O. Both stability and maturity of frass increased significantly with higher inclusion of fresh frass in the larvae's diet. Seed germination (an index related to phytotoxicity) with frass extracts increased from 0.81% (0% fresh frass inclusion) to 43% (100% inclusion), demonstrating a highly significant reduction of phytotoxicity when fresh frass is used in the larvae diet. Finally, this more mature and less phytotoxic frass also had concentrations of pathogenic bacteria within legal limits for agricultural use (*Salmonella* spp. and *Escherichia coli*). BSFL frass can be stabilized and have its quality improved by recirculating it back as a dietary component for the larvae.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Antibacterial potential of fungal bioactive molecules from *Hermetia illucens* gut against *Salmonella enterica* Pullorum

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The mycobiota of the digestive tract of the fly *Hermetia illucens* represents a source of biologically active compounds with potential antibiotic properties, offering promising alternatives to conventional antibiotics. *Salmonella enterica serovar* Pullorum (*S.* Pullorum), responsible for avian typhoid, result in economic losses in the poultry industry, and antibiotic treatments contribute to bacterial resistance. Specific application against this pathogen remains unexplored. The study aims to fill knowledge gaps by highlighting the potential of *H. illucens* fungi as a source of effective antibiotic molecules against *S.* Pullorum. The exploration of the activity on SP of the group of fungi isolated from the fly digestive tract allowed the identification of an active fungus. The fungus belonging to the genus *Aspergillus* was identified with the code HGU11_3. The following step was mass liquid cultivation. Subsequently, an ethyl acetate extract was obtained, and its antimicrobial activity was validated through bioautography. Bio-guided fractionation was carried out using MPLC chromatography. The active fractions were analyzed by UHPLC/MS. Mass spectrometry analysis revealed that the bioactive extract from *Aspergillus* was predominantly composed of dipeptides/cyclopeptides. In conclusion, these findings suggest a potential therapeutic interest of the identified bioactive subfractions, paving the way for future investigations into their biomedical and veterinary applications.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Optimizing plant growth: unveiling the potential of controlled thermal processing of frass as sustainable soil amendment

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The residual organic stream generated from edible insect mass production, commonly referred to as frass, is recognized for its nutrient-rich composition, positioning it as a promising bio-based solution for enhancing plant growth in contemporary agriculture. This study delves into the potential of controlled thermal processing and investigates the impact of black soldier fly (BSF) *Hermetia illucens* L. larvae frass as a soil amendment to enhance its effectiveness as a sustainable source of plant nutrients. In a greenhouse experiment, soil was amended with two different doses (2 and 5 g/kg) of frass, which had been subjected to a heat treatment by heating samples at 70 °C in an oven; untreated; or left unamended (control). The BSF frass originated from two different sources. Plant growth parameters, including leaf area, number of leaves, and shoot dry biomass, were measured to assess the influence of thermal processing on the performance of *Brassica rapa* L. Our findings unveil significant effects thermal processing of frass and dose of application on leaf surface area and dry biomass in *B. rapa*, with higher values observed in frass-amended soil at 5 g/kg compared to the unamended soil and 2 g/kg. Similarly, preheating frass resulted in a higher leaf count per plant. However, regardless of treatment, the number of leaves did not differ between frass doses and sources. The study underscores the potential of controlled thermal processing and optimal dosage in maximizing the agronomic benefits of BSF frass as a sustainable fertilizer for *B. rapa* cultivation. These results offer valuable insights into refining frass application in agriculture. The study emphasizes the potential of thermal processing of BSF frass as a bio-based solution for enhancing *B. rapa* growth. Further investigations to uncover underlying mechanisms represent an intriguing next step towards fostering sustainable agriculture and safeguarding the environment.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Frass extract from *Hermetia illucens* to cultivate pak-choi and water celery

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In a production plant (www.CUBESCircle.de) for the black soldier fly *Hermetia illucens* (L.), the insect frass was tested for its suitability as a horticultural fertilizer. Extracts were prepared from various treated black soldier fly frass residues and tested for their suitability as a fertilizer for two leafy vegetables: *Brassica rapa* var. *chinensis* L. (pak-choi) and *Helosciadium nodiflorum* (L.) W.D.J. Koch (water celery). The extracts were compared with the standard fertilizer Universol® Green as a positive control, either alone or enriched with important nutrients. The extracts were prepared from oven-dried or fresh material. All plants were grown in sand (5% perlite) and fertilized twice a week with the different solutions. Plant quality was analyzed for terpenoid and glucosinolate content using GC-FID and HPLC. The water celery, which was fertilized with enriched extracts from the fresh frass of the black soldier fly, had the highest fresh weight and the highest terpenoid content together. In contrast, the highest fresh weight and the highest glucosinolate content were obtained in pak choi, when the plants were fertilized with the extract from the freeze-dried enriched frass. In both cases, comparable results to fertilization with the commercial fertilizer were obtained. In summary, it can be said that insect frass extracts can be used as an alternative resource for the use of fertilizers.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Black soldier fly frass used for nutrition of vineyards

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Insect rearing residues (frass) are a viable alternative to chemical fertilizers as they contain easily assimilable minerals and chitin which stimulates the immune defences of plants. This study aimed to evaluate Black soldier fly frass (BSFF) as fertilizer and to evaluate its possible dosage for vineyard nutrition. The trial vineyard consists of 700 plants of Pinot Noir variety and Kober 55BB rootstock. BSFF was manually distributed along the row and buried with an inter-row machine. Based on soil and BSSF analyses, different concentrations were obtained from a hypothetical fertilization plan due to meet the nutritional needs of the plants: A (1,400 kg/ha), B (2,100 kg/ha), C (2,800 kg/ha), D (4,300 kg/ha), and E (negative control). To obtain reliable data, 10% of the vines were randomly selected, labelled, and vegetation parameters were sampled from spring to harvest: shoots length, weight of grape bunches and their analytical values (pH, acidity, sugar content, and yeast assimilable nitrogen – YAN). The data were collected in triplicate and Anova test was used for analysis. The highest grape bunches' weight was recorded for treatment B (187.9 ± 30.27 g), followed by A (165.7 ± 19.20 g), C (134.4 ± 23.61 g), E (109.7 ± 23.53 g), and D (70.1 ± 35.54 g). The analysis suggests that using low concentrations of BSFF in treatments A and B is associated with obtaining grapes with higher average weights compared to both the control (p-value < 0.005) and treatment D (*P*-value < 0.001). Additionally, treatment C shows statistically significant differences from both treatment B (*P*-value = 0.012) and D (*P*-value = 0.002). To evaluate the quality of the grapes too analysis of YAN value was done on the grapes during their harvest. The highest YAN value was recorded using treatment B (122.0 ± 7.55 mg/L), followed by C (121.0 ± 17.21 mg/L), A (103.3 ± 3.51 mg/L), D (97.7 ± 3.06 mg/L), and E (86.0 ± 5.57 mg/L) treatments. A, B and C of YAN were statistically different from control (*P*-value < 0.005) and A and D were different from both B and C (*P*-value < 0.005). In conclusion, the use of BSFF seems promising in contributing to the production of grapes with higher weights and improved YAN levels than control.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Insect residues as eco-friendly fertilizers: a study on nutritional effects in tomatoes and microgreens

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In the near future, a rapid increase in insect production is expected, generating huge amounts of frass-based residues. In the context of circular economy and agricultural sustainability, such residues can be used in various ways: as organic fertilizers in the field, replacing mineral fertilizers, or in protected environments such as greenhouses and vertical farms, where they serve as cultivation substrates and sources of macro and micronutrients. This study aimed to evaluate the effect of *Hermetia illucens* (H) and *Tenebrio molitor* (T) frass on the nutritional quality of tomatoes and mizuna and rapini microgreens, in two different production contexts: open field (tomatoes) and growth chambers (microgreens). At commercial maturity, collection and chemical-nutritional characterization of the edible products were carried out. In both tomatoes and microgreens the use of frass did not affect yield or visual quality. In tomatoes, the use of T frass did not result in variations in the following nutritional parameters: colour, Brix, titratable acidity, and major mineral elements (Ca, P, Al, B, Cu, Fe, Zn, Na, and Mn). In tomato samples, the untargeted metabolomics protocol detected 72 metabolites, with variations related to the presence of frass as fertilizer. In mizuna cultivated with H frass, a significant increase in mineral content such as Fe (+28%) and Zn (+147%) was observed compared to the control with peat, with benefits for human health. Furthermore, no contamination by *Salmonella* spp. and *E.coli* or heavy metals (Cd, Cr, Ni, Pb, Hg) was detected in the microgreens. In both production systems, the use of H and/or T frass allowed for yields similar to chemical fertilization, with an improvement in some nutritional traits. It is then possible to guess a large scale application of insect breeding wastes to replace of chemical fertilizer with a positive impact for vegetable productions and the environment. Supported by PRIMA2021 Advagromed project.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Processed black soldier fly products retain antimicrobial peptides active against animal pathogens

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Antimicrobial peptides (AMPs) are small proteins that are produced by organisms as an innate immune response to pathogens. The black soldier fly (BSF) *Hermetia illucens* (BSF) have been found to have over 50 AMPs characterised in its genome, making it one of most promising invertebrate sources of AMPs. In this study, processed and unprocessed BSF samples were evaluated using UPLC G3 QToF mass spectrometer techniques for the presence of AMPs. The samples were further characterized against a library of bacterial pathogens of various taxonomic groups using broth microdilution for determining minimum inhibitory concentration. Results found evidence of peptides in all BSF samples including processed commercial samples. Peptides from the classes Cecropin, Defensins and Attacins were found and relatively quantified in the samples as well as potential peptides never previously found in BSFL. In vitro results indicate the BSFL samples had an antimicrobial effect against pathogens such as Salmonella and Staphylococcus, most likely attributable to the AMPs found. These results indicate that commercial BSFL products, even when subject to the heat and pressure of processing, retain AMPs at significant enough quantities to inhibit animal pathogens.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Chitosan and melanin from the black soldier fly

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The recent years have seen a push across many industries for sustainable and naturally-derived biomaterials. While black soldier fly farming has typically been advocated for the production of feed and fertiliser, the side streams generated from this system are rich in chitin and melanin, which could be refined, derivatised, and commercialised at an attractive value. The production of these biomaterials is scalable and solves a major problem with consistency, due to the use of a commercially-relevant raw material from a single species. In this talk, we briefly introduce the opportunity for chitosan and melanin production from the black soldier fly, describe the inherent advantages of these biomaterials over traditional sources, and explore both existing and novel applications in which they may be utilised. Elemental analysis, potentiometric titration and viscometric measurements were used to characterised chitosan. Analyses revealed that BSF chitosan was of a low molecular weight (<100 kDa), had a high deacetylation degree (85-90%), and a low ash content (<0.5%). Investigations of BSF melanin revealed a characteristic eumelanin nature, with peculiar solubility in both water and organic solvents depending on processing technique. BSF melanin was further investigated in bone regeneration scaffolds, energy storage devices and humidity sensors, where it enabled cell adhesion and proliferation, had promising charge storage, and allowed for fast response/recovery time respectively.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Valorisation of municipal solid waste through *H. illucens* larvae to produce bioplastics for the electronics industry

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The valorisation of the organic fraction of the municipal solid waste (OFMSW) using black soldier fly (BSF) larvae has proven effective. However, the efficiency of the bioconversion process and the quality of the resulting insects may be affected by the variable composition of the substrate. Furthermore, national regulations often limit the use of insect protein for animal feed production to larvae reared on microbiologically and chemically safe substrates. This study aims to address these two issues by (1) investigating the role of the midgut – responsible for nutrient digestion and absorption – in the capability of the larvae to exploit OFMSW with varying chemical compositions, and (2) exploring the suitability of proteins isolated from BSF larvae reared on OFMSW for producing value-added bioplastics for advanced applications, other than food and feed. Our findings indicated that, despite differences in nutrient content among the tested feeding substrates, the larvae were characterized by similar protein (approximately 36%) and lipid (44%) content. This result is guaranteed by post-ingestion regulatory mechanisms at the midgut level, modulating gene expression and digestive enzyme activity, that enable larvae to compensate for variations in nutrient composition of the substrate. After defatting with petroleum ether, insect proteins were extracted through solubilisation at pH 11.0 and precipitation at pH 4.0, and subsequently processed for the production of bioplastic films. By incorporating CCB/SP as a conductive nano-filler into BSF proteins, we developed high-performing electro-conductive nanocomposite films that can be applied as matrices for flexible and printed electronics. This result support the creation of circular supply chains to produce protein-based materials with significant technological value through bioconversion of the OFMSW. The study was funded by Fondazione Cariplo (2020-0900) and MUR (2020ENH3NZ).

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Hi-Tech project: *Hermetia illucens* biofactory: from waste to high value technological products

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Growing demand for eco-friendly products has sparked research into various materials and applications. Applying the full circular economy principle, HI-Tech project aims to use black soldier fly larvae (BSFL) to bioconvert organic waste, into lipids, proteins, and chitin to produce bio-based materials for technical applications. The novelty of the HI-Tech project relies on the use of the components extracted from BSFL not for feed purposes, but to produce bio-based materials for technical applications, including biosurfactants, bioplastics, polymers, and pharmaceutical products. Indeed, information in this field is currently limited and more research is demanded. In Hi-Tech, BSFL are reared on local organic substrates (former products containing meat and fish and catering waste) and products (larvae, puparium, dead flies) assessed for their chemical composition (crude protein, lipids and chitin) and their safety (microbiological and heavy metals analyses). Each fraction can be valorised based on its chemical value: (1) Proteins are used as protein-based bioplastics/biocomposites or natural dyes; (2) Lipids, due to their fatty acid profile rich in lauric acid, are transformed into 100% biobased and biodegradable sugar esters with application as surfactants; (3) Chitin is depolymerized to N-acetylglucosamine and/or glucosamine, that are new N-containing platform molecules for the synthesis of chemicals, pharmaceuticals and polymer precursors (supported by PRIN 2022).

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

House cricket frass used in biofuel production

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The utilization of insects for food generates significant organic waste in the form of excreta, known as frass, which can accumulate to thousands of tons annually. Exploring the potential of utilizing insect frass for bioethanol production mirrors the practice with conventional livestock manure. House crickets were selected for this study due to their existing use in the food industry. The crickets were cultivated under controlled conditions (32 °C, 70% moisture, 8-hour photoperiod) and fed a commercial cricket diet. Frass was collected throughout their life cycle, sterilized at 121 °C and recognized as a source of proteins and polysaccharides. Initially different digestion processes were tested, namely enzymatic digestion with cellulases (50 °C, 10% enzyme/substrate, pH = 5, 48 h), enzymatic digestion with proteases (50 °C, pH 6.8, 24 hours), acidic digestion (50% sulfuric acid) and alkaline digestion (20% sodium hydroxide) during the sterilization. The treatment with proteases and the combination of acidic digestion with the treatment with cellulases led to the production of FAN and free sugars at 5 g/L and 30 g/L, respectively. The growth on frass was confirmed for several yeasts, including Saccharomyces cerevisiae (commercial and from municipal waste), *Candida parapsilosis* isolated from frass, and *Candida kefyr* from tomato pomace. For the production of bioethanol, frass was hydrolyzed with a combination of acidic treatment and enzymatic digestion with both cellulases and proteases and the chosen yeast was Saccharomyces cerevisiae. The substrate was frass together with molasses at concentrations of 0 to 60 g/L for 48 hours. At the end of the process, the sugar consumption exceeded 80%, resulting in ethanol yield of 12.56 g/L and 21.85 g/L without and with molasses addition of 60 g/L, respectively. It was concluded that insect frass holds potential for bioethanol production, with enhancement observed through the addition of beet molasses.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Untargeted metabolomics of ripened tomato fruits: insect frass fertilizer effects

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In this study, insect frass (*Tenebrio molitor*, TM) and poultry manure were tested as organic fertilizers in an open field trial conducted during the 2023 spring-summer season for cultivating the local variety of tomato (*Solanum lycopersicum* L.) cv "Regina." Nine treatments (T1-T9) were established in a randomized block experiment with three replications. The treatments included: 100% conventional mineral fertilization (control T1), 100% TM frass (T2), 100% organic fertilizer (T3), 100% poultry manure (T4), 75% mineral + 25% TM frass (T5), 50% mineral + 50% TM frass (T6), 75% mineral + 25% poultry manure (T7), 50% mineral + 50% poultry manure (T8), and no fertilization (T9). Ripened tomatoes were harvested from each treatment, pooled, immediately freeze-dried, and stored for successive metabolomic analysis. An untargeted metabolomics protocol, utilizing HPLC-HRMS with a Thermo Scientific Orbitrap Fusion coupled to an Ultimate 3000 chromatograph, was applied. Samples were extracted using polar solvents, and the obtained solutions were separated on a reverse-phase column. The hypothesized compounds were annotated after HRMS/MS fragmentation in both positive and negative ion modes. In total, 72 metabolites were detected in all the samples. Concerning the TM frass treatments (T2, T5, and T6), T2 and T6 apparently reduced, while T5 increased the expression of annotated compounds when compared to the negative control treatment (T9). The integrative approach of HPLC-HRMS in untargeted metabolomics facilitates an understanding of the complex interactions between fertilization and tomato crop biochemistry, contributing to the enhancement of crop quality, yield, and sustainability in agricultural practices. Supported by PRIMA2021 Advagromed project.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Analysis of coconut rhinoceros beetle (*Oryctes rhinoceros*) larval frass as plant fertilizer

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Animal manure has a long history of use as a soil amendment. Recent data suggests supplemented with frass at composting. The coconut rhinoceros beetle (*Oryctes rhinoceros*) is a pest species that could potentially be collected or reared as food or feed. Larvae produce prodigious amounts of waste, and beetle breeders in certain areas informally give larval manure to farmers as fertilizer. This study examined the manure of artificially-reared, cocopeat-fed *O. rhinoceros* as a soil amendment. Analyses of the frass included NPK levels, C:N ratio, levels of polysaccharide digestions, particle size distribution and shape, and soil microbiota inventory. To determine the effect of fertilization with beetle frass on plant growth, Arabidopsis thaliana were grown on soil at 0, 20, or 40% by weight, and their elemental concentrations measured. Based on nitrogen, mineral, and pH levels, the frass is suitable for use as a fertilizer without composting, with a mean C:N ratio of 22.7, meaning the larvae had biocomposted their substrate. The frass had uniform and continuously distributed particles mostly below 68 μm in diameter. Neutral sugar measurements and NMR spectra suggested low lignase activity but significant digestion of hemicellulose. Plants growing on soil containing greater percentage frass were noticeably larger, with more biomass and earlier flowering, even though the control soil was an optimal medium with moderately more nutrients than the frass mixtures. These observations suggest a non-nutritive benefit of the larval frass, possibly linked to the microbiome. Future research will need to confirm and identify the compound responsible for the growth stimulatory effect of beetle frass on plants.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Evaluation of insect frass as a nutrient supplement for macroalgae cultivation

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Substituting significant quantities of chemical fertilizers with nutrients derived from waste or residual streams is crucial for enhancing the sustainability, cost-effectiveness, and integration of macroalgal production into a circular bioeconomy. This study investigated the hydrothermal extraction of nutrients from mealworm (*Tenebrio molitor*) frass explicitly tailored for *Ulva meridionalis* cultivation. Insect frass, known for its nutrient-rich composition, underwent high-pressure steam treatment to extract soluble nutrients, including ammonia and phosphate compounds. The extracted nutrient solution represents a potential source for *Ulva meridionalis* cultivation. Various parameters of the hydrothermal extraction process, such as temperature (120 °C, 140 °C, 160°C, and 180 °C), biomass-to-water ratio (2.5%, 5%, 7.5%, and 10%), and residence time (15 min, 30 min, 45 min, and 60 min), were explored to optimize extracted nutrient concentrations. The results indicated that high concentrations of 696.82 mg/L ammonia and 854 mg/L phosphate were achieved at 160 °C for 45 minutes with a 7.5% biomass-to-water ratio. Subsequently, the extracted nutrients were utilized to cultivate *Ulva meridionalis*, resulting in similar growth and biochemical contents as when grown in a control medium based on commercial fertilizers. The findings reveal hydrothermal extraction effectively enhances nutrient availability for *Ulva meridionalis* cultivation, improving growth and biochemical contents. This study contributes to developing sustainable practices in macroalgae cultivation by harnessing insect frass as a valuable nutrient source through hydrothermal extraction techniques.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Synthesis and characterization of functional peptides via subcritical hydrothermal hydrolysis of YML defatted meal

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In recent years, the escalating global population has intensified the demand for food, prompting the exploration of insects as a promising new food source. Insects offer substantial nutritional value, with bioactive peptides derived from their proteins recognized for their potential to regulate physiological processes and exhibit resistance against oxidative properties. This study investigated the utilization of agricultural by-products to nourish mealworm larvae, followed by employing an insect transformation platform to produce functional peptides. Preceding subcritical water hydrolysis, mealworm larvae undergo harvesting, microwave-drying, defatting, and freeze-drying processes. Subcritical water hydrolysis is conducted with biomass ratios of 1:9, 2:8, and 3:7 (w/v) at 180 °C, with subsequent preliminary analysis of the resulting hydrolysate. The findings revealed a correlation between lower solid content, enhanced hydrolysis degree, and increased yield. Notably, peptides of low molecular weight obtained at a 1:9 (w/v) ratio exhibit the highest yield at 40.97%. These results elucidated the optimization of hydrolysis parameters, facilitating the production of functional peptides from insect frass for diverse applications in the food and biotechnology industries.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Evaluation of insect frass as soil fertilizer for lupin and faba beans cultivation in field trials in Greece and Morocco

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Insect frass, i.e. the leftover of the insect rearing process, is the major by-product of insect production facilities in volume and weight. Recent research has shown the potential of frass as an effective soil amendment and organic fertilizer that can greatly improve crop growth. However, results from field trials showing the plant growth promoting effect of frass are still scarce. In this context, the objective of the present study was to evaluate frass from the two most massively produced edible insect species, i.e. the yellow mealworm, *Tenebrio molitor*, and the black soldier fly, *Hermetia illucens*, as soil fertilizer for lupin and faba beans cultivation. To this end, field trials were carried out in Greece with two white lupin (*Lupinus albus*) varieties, i.e. Nelly and Tennis, using *T. molitor* frass for soil fertilization, in two experimental fields, one in northern and another in central Greece. Three frass rates (i.e. 0.1, 0.5, and 1% wfrass/vsoil) were tested, in comparison with negative (no frass) and positive (chemical fertilizers typically used by local producers) controls. Crop growth and functional parameters, along with lupin yield were assessed. Similarly, field trials were performed in Morocco, with faba beans (var. *Sbai beldi*) using *H. illucens* frass. Based on the frass N content and the faba beans N fertilization requirements, six treatments were evaluated (negative and positive controls, 1,087.5, 2,175, 4,350 and 8,700 Kg frass/ha) in terms of crop performance. This work aims to further unfold the potential of insect frass as soil fertilizer and boost its valorization in organic agriculture. Financial support for this research has been provided by PRIMA, a program supported by the European Union, under grant agreement No 2231, project CIPROMED (PRIMA Call 2022 Section 1 Agri-food IA).

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Application of insect meal as the alternative nitrogen source in the cultivation of *Ceriporia* sp. for artificial leather

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The rising demand for sustainable and eco-friendly materials has spurred the investigation of alternative sources for artificial leather production. This study delves into the feasibility of using insect meal as a substitute nitrogen source in the cultivation of Ceriporia sp. for artificial leather production. Ceriporia sp. is a fungus renowned for its ability to generate leather-like materials. Various concentrations of insect meal are integrated into the growth medium to evaluate their impact on Ceriporia sp. growth and the quality of artificial leather produced. The results reveal that insect meal supplementation significantly enhances the growth rate and biomass yield of Ceriporia sp., consequently improving the texture and durability of the resulting artificial leather. This study highlights the potential of insect meal as a sustainable nitrogen source for Ceriporia sp. cultivation, presenting a promising avenue for the development of eco-friendly alternatives in artificial leather production.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Progress on the efficient grease synthesis by organic waste bioconversion employing black soldier fly

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Global energy and food shortages are major challenges that affect socio-economic activities and threaten sustainable development. Large quantities of organic waste are generated worldwide and are not properly managed, especially in low- and middle-income countries. Biorefineries, as the main technology of the circular bioeconomy, can maximize the use of biomass, curb environmental pollution during bioenergy conversion, and provide a wide range of bioproducts including biofertilizers, biochemicals, and bioenergy services to the socio-economy through integrated and effective management techniques. Under ideal conditions, insect farming has slightly higher reported values of up to 70% protein recovery and 30% heat recovery. While the first generation biodiesel process relied mainly on catalyzed transesterification, 94% of the biodiesel produced by black soldier fly larvae (BSFL) is produced by non-catalyzed transesterification. This increases the sustainability of biodiesel production and reduces the energy input in the production process. We not only optimized the reaction parameter scheme for biodiesel production using BSFL, but also revealed the basis for rapid fat accumulation in BSF at the molecular level using transcriptome sequencing and analysis. Characterization of fat accumulation models in BSF was developed to regulate enzymes in lipid biosynthesis. In addition, molecular manipulation of the important genes ACC and FAS after detecting their expression levels in different growth periods and tissue sites in BSF facilitated in-depth studies of lipid accumulation in BSF. In future, more research should be conducted based on investigating the role of proteins and genes responsible for lipid biosynthesis in BSF. It may pave path to improve the lipid quantity and quality of *H. illucens* especially for biodiesel production.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Mycorpotein production from insect frass using edible fungal mycelium in liquid state fermentation

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Insect frass has a high nitrogen concentration in the form of protein, uric acid, chitin, and other chemical species. The recovery of the organic nitrogen in the form of high-quality protein would increase the sustainability of insect production. Fungi are able to digest these nitrogen species and incorporate them into protein. Three strains belonging to different fungal species were cultured using frass *Tenebrio molitor* and *Hermetia illucens* as nutrients. Preliminary results show that supplementation with malt extract allows the strains to grow on dry *H. illucens* frass, but dry frass still need to be optimized due to the presence of inhibitors that originated during drying. Wet samples succeeded and will be further researched. Cultures in liquid medium using uric acid and frass from *T. molitor* were performed with and without carbon and nitrogen supplementation to verify the ability of each strain to exploit these compounds. To assess the nitrogen source exploited by each fungal strain, different enzymatic activities were spectrophotometrically quantified using specific substrates: proteases, uricases, chitinases, and laccases, the latter implied in the degradation of complex phenolic compounds that contain organic nitrogen. In the case of *T. molitor* frass, the edible fungi *Flammulina filiformis*, *Lentinula edodes*, and *Pleurotus ostreatus* successfully grew on frass in liquid-state fermentation. Uric acid can be used as a nitrogen source by all strains but not as a carbon source. The highest mycoprotein harvest was obtained with *P. ostreatus* (23% of Frass transformation measured as dry weight of mycelium/d.w. frass after 35 days), and this strain showed a high activity of uric acid and phenolic compound degradation. The other two strains reached 20% of transformation. *F. filiformis* showed an active exploitation of chitin and protein, while *L. edodes* evinced a degradation profile more specialized in protein and uric acid. All the differences are statistically well supported, showing that different strains exploit frass nitrogen differently, paving the way for a combined strategy of sequential frass transformation into mycoprotein combining edible fungal mycelia.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Conversion of insect refinery product glycerol into electricity by microbial fuel cells

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Glycerol is a by-product of the refining process of insects to produce biodiesel, which can be converted to a valuable chemical product. However, the gradual oversupply of glycerol and the cost of refining crude glycerol make the disposal of glycerol a potential challenge. Microbial fuel cell (MFC) is a technology that uses a substrate as a fuel and provides microorganisms to convert it into electrical energy. However, immature electrode materials are a limitation for MFC power generation. In this study, heat-treated carbon cloth, which is porous and rich in hydrophilic oxygen-containing functional groups, was used as the anode material for MFC, with the aim of enhancing the efficiency of glycerol conversion to electrical energy. In this study, heat-treated carbon cloth, which is porous and rich in hydrophilic oxygen-containing functional groups, was used as the anode material for MFC, with the aim of enhancing the efficiency of glycerol conversion to electrical energy. The results showed that the MFC system achieved high coulombic efficiency (43.49%) and high normalized energy recovery (NER) (0.13 kWh/kg COD) with a power density output of up to 21 mW/m^2. Changes in the anodic phase structure of the MFC were also observed, with a significant increase in the relative abundance of electroactive microorganisms after 12 days of operation of the MFC system. A biotoxicity test of *Vibrio fischeri* was used in this study to observe the toxicity of the anode solution after the operation of the MFC system, which showed that the anode solution was still toxic to *Vibrio fischeri* even after 100% removal of glycerol, indicating that the glycerol was not only converted into electrical energy, but also had the potential to be generated as a by-product.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Improving the electrodes of microbial fuel cells using insect waste to enhance glycerol utilization and power production

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Insects contain many fats, increasing the use of biomass energy from insect refining. However, the large amount of insect waste produced pollutes the environment. Moreover, the process of refining insects into biodiesel increases the production of glycerol as a by-product, and the accumulation of large quantities of glycerol also poses an environmental threat. Therefore, upgrading glycerol and reusing insect waste is a priority issue. There are many alternatives for degrading glycerol, among which microbial fuel cell (MFC) is an environmentally friendly and cost-effective technology. Biochar (BC) made from insect waste (shells) has multifunctional groups and porous structure, improving the stability of power generation and degrading more glycerol. This study adds glycerol as a substrate for microorganisms in the anode tank, and uses insect shells to fabricate the biochar electrodes to investigate the microbial growth, electricity production and degradation efficiency of MFC systems with various configurations of biochar electrodes. The MFC anode and cathode configurations consisted of (1) biochar placed at the anode and cathode (MFCBC/BC); (2) biochar placed at the anode and carbon cloth (CF) used at the cathode (MFCBC/CF); and (3) carbon cloth placed at the anode and cathode (MFCCF/CF). The results showed that the MFCBC/CF configuration had the highest microbial growth, and the voltage output of this configuration was 1.81 and 1.47 times higher than those of the MFCBC/BC and MFCCF/CF configurations, respectively. The results of this study demonstrated that the biochar electrodes are favourable for microbial growth and improved conductivity.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Enhancing agricultural productivity through industrial insects frass utilization

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Research report explores the potential of industrial insects as a source of frass, a unique organic fertilizer that can significantly benefit agricultural practices when properly managed. The utilization of frass offers a sustainable and environmentally friendly approach to enhance soil fertility and crop productivity. Furthermore, this research delves into the importance of remote sensing, soil analysis, and additives in optimizing agricultural practices based on Frass to achieve increased crop yield and improved crop resistance to stress. By integrating these technologies and practices, farmers and agricultural stakeholders can harness the full potential of frass and industrial insects to foster a more resilient and productive agricultural system. Overall, this research serves to underscore the critical role of industrial insects and frass in sustainable agriculture, offering insights into how these resources can be harnessed to promote agricultural sustainability and productivity and new revenue of income industrial farmers.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Positive effect of BSF fertilizer on the spinach crop: an advancement in agriculture

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Black soldier fly (BSF) frass and its derived products have gained attention worldwide in animals and agricultural applications. Numerous studies have revealed that frass can supply soil with essential macro and micronutrients, organic matter, microorganisms, and chitin that reduce disease, modify soil microflora, and influence plant behaviour. However, the information on commercial application of frass as a fertilizer is limited. This study aims to explore the use of frass as an organic fertilizer alone or with mineral fertilizer on spinach crop. The fertilizer application was done as per the recommended dose of fertilizers (80:60:60 N:P:K) to seven treatments in three replications of RBD pattern: T1 or Treatment 1 (no fertilizer), T2 (other organic), T3 (100% mineral), T4 (100% BSF), T5 (25% BSF + 75% mineral), T6 (50% BSF + 50% mineral), T7 (100% mineral + additional 50% BSF) and the calculation was done as per the N:P:K ratio present in the respective fertilizers. Seeds were procured from the IARI-ICAR, New Delhi, and standard procedure was followed for spinach cultivation. 50% of the fertilizer was applied during sowing, and the remaining 50% after second and third harvests, followed by irrigation. Several basic morphological features, such as crop yield, plant height, leaf number, leaf width, plant density, etc., were observed and data collected at 15-day intervals. ANOVA test was performed with 95% confidence interval to do a statistical analysis on the data set derived from all treatments. The results show that T7 has the highest crop yield, accounting for 16.8% of the total crop production across all treatments, followed by T3 and T4, which are almost similar. Among the other morphological parameters, T4 performed well compared with other treatments. It is only 7.66% and 8.02% less than the T7 (highest) in plant height and leaf width, respectively; on the other hand, it is 4.65% higher in leaf number than the second highest T7. Based on data from this comparison study, BSF fertilizer performs well due to its unique characteristics, and it may be used as an alternative to excessive mineral fertilizers; however, detailed research is required prior to commercial application for a variety of crops.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Utilizing black soldier fly oil in the preparation of artificial leather made from spent stems of golden needle mushroom

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This study explores using golden needle mushroom roots as natural fiber materials for developing artificial leather. Bio-leather is synthesized using black soldier fly oil, renowned for its high saturated fat content. The bio-leather composition consists of golden needle mushroom roots, tannic acid, black soldier fly oil, glycerin, and sodium alginate. The final processing step involves applying a mixture sprayed with 1% calcium chloride. The process entails a cross-linking reaction between sodium alginate and calcium chloride. The optimal ratio is determined through contact angle, tensile strength, and puncture resistance measurements by varying the proportions of glycerol, sodium alginate, and tannic acid. Artificial leather produced with 20 times glycerin, 2wt% sodium alginate, and 3wt% tannic acid exhibits superior flexibility, tactile sensation, and tensile strength. The inclusion of glycerin further enhances the leather's flexibility. This innovative method reduces reliance on animal leather and mitigates environmental impact. The resulting product is biodegradable, offering sustainable alternatives for the leather industry. Thus, this study contributes to promoting environmentally friendly materials within the leather industry, facilitating advancements towards sustainability.

INSECT PRODUCTS AND FRASS FOR NON-FOOD APPLICATIONS, BIOFUELS AND BIOACTIVES

Using different strains of bacteria to ferment insect frass to develop organic liquid fertilizer

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This study investigates the utilization of various bacterial strains to ferment insect frass (by-products of mealworm excrement) and agricultural waste to develop organic liquid fertilizer. Insect frass and agricultural waste present valuable sources of nutrients and organic matter, offering potential alternatives to conventional fertilizers. Through microbial fermentation processes, diverse bacterial strains are employed to break down the organic compounds in insect frass and agricultural waste, enriching the resulting liquid fertilizer with essential nutrients and bioactive compounds. The study explores the efficacy of different bacterial strains, including Trichoderma, *Bacillus subtilis*, and *Bacillus amyloliquefaciens*, in optimizing the fermentation process and enhancing the nutrient content and bioavailability of the liquid fertilizer. By harnessing microbial fermentation, this study aims to promote sustainable agricultural practices by providing a viable solution for organic fertilizer production while reducing reliance on synthetic fertilizers. Elemental analysis revealed the effective dissolution of critical nutrients such as nitrogen, with the liquid fertilizer containing 0.15% nitrogen subsequently utilized for vegetable cultivation. The vegetables' growth rate and leaf chlorophyll levels were evaluated to demonstrate efficacy. Results indicated that the liquid fertilizer fermented with *Bacillus amyloliquefaciens* was the most effective, outperforming commercial organic fertilizers by increasing yields by 20% and chlorophyll content by 10%. These findings contribute to developing environmentally friendly agricultural solutions and underscore the potential of insect frass and agricultural waste as valuable resources for organic liquid fertilizer production.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Using pool-sequencing to evaluate the impact of body weight selection on the genome of black soldier fly

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Black Soldier Fly (BSF, *Hermetia illucens*) larvae can convert low-grade organic materials into high-quality products. Its use has increased a lot on a global scale in recent years. Production efficiency is an essential criterion for the industry, where genetics can play an essential role. Since 2019, in a collaborative effort between Protix and Hendrix Genetics, a genetic improvement program was set up to increase larval body weight in BSF. After 16 generations of selection, genetic improvement translated into an increase of 39% of the body weight under factory production settings. This study aimed to assess the associated impact on the genome. For up to 12 generations, a control line without selection was maintained in the same conditions as the selection line. Genetic material was available for the base population (38 samples) and for both lines at generation 8 and generation 12 (30 and 90 samples, respectively). A pool-sequencing approach with 30X coverage was used per generation/line as a relatively cheap and accurate approach to obtain genomic data. After quality control on the pooled sequences, variant calling and filtering led to the identification of 18 million SNPs (single nucleotide polymorphism). PoPoolation2 toolkit was used to derive SNP frequency estimates from the pooled sequences. The degree of differentiation of populations was computed using Wright's Fixation index, applied on sliding windows of 10 kb. The average degree of differentiation after 12 generations compared to the base population was 0.070 on the selection line, while it was 0.054 on the control line. When looking at the distribution among the genome, with a threshold of 0.25, two regions were detected on chromosome 6 for the control line. These regions might be related to the adaptation to the environmental conditions. These two regions were also detected for the selection line but with fixation in the opposite direction. On top of that, three other regions were detected on chromosomes 2, 3, and 4. These regions are likely to play a role in the genetic architecture associated with body weight of BSF.

INSECT BREEDING, GENETICS AND BEHAVIOUR

UVB exposure as an immunostimulant of the yellow mealworm: a transcriptomic approach

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The yellow mealworm (*Tenebrio molitor*) synthesizes vitamin D_3 *de novo* when exposed to ultraviolet B (UVB) radiation. Despite the well-known role of vitamin D in metabolic and immunological processes in vertebrates, its significance in insect physiology is not well understood. Groups of 200 mealworms were either UVB-exposed or UVB-unexposed (control) for two weeks after which they were subjected to an entomopathogenic fungus (*Beauveria bassiana*) to evaluate the potential of vitamin D as an immunostimulant. Survival, and weight were determined before the fungal challenge (D0), and after 7 (D7), and 14 days (D14). Moreover, on these days subsamples were taken for differential gene expression analysis. Exposure to UVB did not affect survival, but mealworms in the control group had a 1% higher average weight at D0 and a 16% higher weight at D14. The transcriptomic analysis at day zero revealed significant overexpression of the Toll pathway – a key signaling pathway mediating bacterial, fungal, and viral immunity in insects. Furthermore, the expression of anti-microbial peptide (AMP) genes, including *Tenecin 4*, *Coleoptericin B*, and *Attacin C*, and *Defensin-like* was higher in the UVB-exposed group compared to the control at D0, but not at D7 or D14. This suggests a transient, yet significant enhancement of the mealworm's innate immune response following UVB exposure. Although this did not persist at D7 and D14, the observed positive modulation is noteworthy. These findings are relevant for our understanding of insect immunology, and might find application in commercial rearing facilities combating certain pathogens. Further studies are needed to determine whether increased AMP gene expression from sustained UVB exposure translates to increased protection against pathogens such as *Beauveria bassiana* and beyond.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Unravelling the link between diet-induced phenotypic plasticity and reproductive strategies in black soldier fly adult

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The black soldier fly (BSF, *Hermetia illucens* L.) plays a crucial role in sustainable waste management through its ability to convert bio-waste into biomass during its larval stage. While much research has focused on larval diet and growth, the impact of larval nutrition on adult BSF reproductive traits remains less explored. Adult reproduction is essential for maintaining BSF populations and their efficiency in bio-waste management. Investigating the effects of various larval diets on phenotypic plasticity in adult male and female BSFs offers valuable insights into their reproductive mechanisms and strategies. This study presents a comprehensive investigation into phenotypic plasticity in adult BSFs, focusing on sex-specific plastic responses and adaptive strategies shaped by larval diets. We extensively examined traits including adult body size, reproductive organ development, sperm length, mating behaviours, and life history across different diets. Our findings reveal notable differences in phenotypic plasticity levels between male and female BSFs in various morphological aspects, particularly with females showing increased plasticity in reproductive organ parameters. Furthermore, distinct plasticity patterns were observed in the allometric growth and weight ratio of reproductive organs between sexes. Notably, diets that led to longer male lifespans also prompted earlier male emergence. This discovery highlights a strategic interplay between lifespan and protandry degree variations, aimed at maximizing the overlap of male and female lifespans, thereby enhancing mating opportunities and success in diverse environmental conditions. Additionally, our results reveal behavioural adaptive strategies in mating, where diets producing smaller adults, reproductive organs, and shorter sperm correlated with enhanced mating performance. This research enhances our understanding of BSF biology, particularly the complex interactions between nutrition, development, and reproductive strategies, and has significant implications for their sustainable cultivation and application in insect bioconversion industries.

INSECT BREEDING, GENETICS AND BEHAVIOUR

What complete mitochondrial genomes tell us about the evolutionary history of the black soldier fly, *Hermetia illucens*

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The black soldier fly (BSF) *Hermetia illucens* is a cosmopolitan fly massively used by industrial companies to reduce biowaste and produce protein and fat for poultry and aquaculture feed. However, the natural history and the genetic diversity of the BSF are poorly known. Here, we present a comprehensive phylogeny and time tree based on a large dataset of complete mitochondrial genomes better to understand the evolution and timing of the BSF. In this study, we analyzed 677 CO1 sequences derived from samples found all over the five continents, leading us to discover 52 haplotypes, including ten major haplotypes. This worldwide cryptic genetic and genomic diversity is mirrored at a local scale in France, in which we found five major haplotypes sometimes in sympatry. Phylogenetic analyses of 60 complete mitochondrial genomes robustly resolved the phylogeny of the major BSF haplotypes. We estimate the separation events of the different haplotypes at more than 2 million years for the oldest branches characterizing the ancestral split between present North American lineages and the other highly diverse south-central American clades, possibly the following radiation beyond the isthmus of Panama northwards. Our data confirm that this North American lineage ultimately gave birth to almost all commercial BSF stocks that participated in the worldwide BSF dissemination through farm scapements. Our data resolve the phylogenetic relationships between the major lineages and give insights into the BSF's short and long-term evolution. Our results indicate that commercial BSF stock's genetic and genomic diversity is very low. These results call for a better understanding of the genomic diversity of the BSF to unravel possible specific adaptations of the different lineages for industrial needs and to initiate the selection process.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Let *Hermetia illucens* free (from hunger and discomfort)! Welfare evaluation on larval stage

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Welfare evaluation is raising concerns about farmed animals, where the 5 Freedoms of Brambell's report (5F) are applied. Despite no guidelines for insects yet, the International Platform of Insects for Food & Feed (IPIFF) recommends applying these principles. Insect welfare during the rearing can be indirectly evaluated considering performance parameters, as suitability indicator of the conditions applied. Black soldier fly (*Hermetia illucens* L., BSF) is increasing in popularity as innovative feed and large-scale farming is expected to grow. This study determined the best dietary regime (T1), feeding rate (T2), rearing density (T3) for BSF larvae (L), integrating 5F into the rearing process. Small-scale (100 L in T1 and T2, 320-960 L in T3) and large-scale (2,000 L in T1 and T2, 3,045-9,135 L in T3) experiments were set-up, four replicates for each treatment. We evaluated performance parameters (growth rate, FCR, final larval weight, substrate reduction, survival rate) and BSF chemical composition. In T1, vegetarian (V), carnivorous (M) and omnivorous (O) diets were compared, together with a control (C). Three feeding rates (T2: 50, 100, 200 mg/day/larva) were evaluated, as well as rearing density values of 5, 10, 15 larvae/cm^2 (T3). The data were elaborated using one way ANOVA and Tukey HSD, or Kruskal-Wallis rank sum test and Dunn test. The survival rate did not registered significative differences, but the omnivorous diet was the most efficient for BSF analyzing growth parameters (growth rate +20% and +9% if compared to V and M diets, respectively). Optimized rearing can be achieved by applying a feeding rate of 100 mg/day/larva (FCR -8% and -40% if compared with O50 and O200, respectively) and with density of 10 larvae/cm^2 (final larval weight +7% and growth rate +10% if compared with O15), ensuring efficiency and welfare of the insect. This holds a key role from an industrial perspective, considering the future development of the sector.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Phenotypic and genetic response to artificial selection in the black soldier fly, *Hermetia illucens*

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Artificial selection has dramatically shaped the genomes of many species resulting in phenotypic diversification across the tree of life. In agriculture, the rising insect livestock industry has shown great potential for artificial selection due to large population sizes and short generation times. The black soldier fly, *Hermetia illucens*, has been the focus of this industry over the past decade due to its efficient bioremediation and nutritional properties. Recent studies have identified significant genetic diversity in *H. illucens* populations around the globe. However, capturing and shaping this diversity for optimal industrial application requires further understanding on the phenotypic variation within strains. Here, we genome sequenced domesticated populations of *H. illucens*and explored phenotypic variation within and between strains across several diets. Our experimental assays of domesticated *H. illucens* revealed substantial variation both between and within strains across several life-history traits, suggesting that there may be benefits to using specific strains for waste applications. Exploring genetic and phenotypic variances within strains and families enabled estimates of heritability suggesting several avenues for genetic improvement through artificial selection. Using this information, we performed an evolve and resequencing study for increased pupal size. Response to selection was strong across three independent replicated experiments yielding an increase of 17% in pupal body size. Body size showed complex interactions with life-history traits vastly improving larval biomass yields (+16%) and growth rates (+19%) over nine months. However, increasing pupal body size appeared to reduce the development time of female pupae by -6%, revealing a previously undetected trade-off. Genomic sequencing of 290 individuals provided an initial set of seven candidate genes controlling body size in *H. illucens*. This work highlights the importance for genetic and phenotypic characterisation of *H. illucens* strains to be incorporated into insect farming practices.

INSECT BREEDING, GENETICS AND BEHAVIOUR

A wild black Soldier fly (*Hermetia illucens*) assembly: signs of adaptation and selection

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The black soldier fly (BSF, *Hermetia illucens*) industry is emerging as a promising solution to food security challenges, yet it lags behind traditional livestock in terms of genetics research and infrastructure. This study aims to bridge this gap by sequencing the genomes of wild North American black soldier flies, which have been underrepresented in existing population surveys, as well as those from laboratory and commercial colonies. Our analysis showed evidence of recent gene expansions in families associated with heavy metal transport and tolerance, which could indicate an adaptation to anthropogenic waste. We identified two new regions on chromosome 5 showing patterns of selection, in addition to the previously recognized region on the same chromosome. The first spanned 780 kb, containing 8032 SNPs and seven protein coding genes, 3 of which were associated with insulin signaling, metabolism, and fat production, which could be a response to diet shifts in captivity or the result of artificial selection for larger, faster growing larvae. The second segment examined was 1.4 Mb in length and contained 4,491 SNPs. Within this region, four out of the five identified genes were co-located and encoded proteins predicted to be involved in cuticle formation. These proteins exhibited highly similar sequences, suggesting they may have arisen through gene duplication. This study advances our understanding of BSF genetics, identifying potential adaptations and targets for selective breeding, which can improve their utility and resilience in insect agriculture.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Adult BSF behavior for industry: homogenous cohorts to increase production

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Adult behaviour is a growing area of interest for black soldier fly, *Hermetia illucens*, mass-rearing operations, as it is a realm of inquiry that remains largely untouched especially compared to larval biology. One of the most important questions for companies is how top-down decisions in facility, production, or cage-design can impact fertile egg production. This presentation extends upon a line of research that has investigated the effects of aging and cohort heterogeny in black soldier fly mating populations. Laboratory-scale experiments within an enclosed greenhouse examined the consequences of utilizing relatively homogenous (a) 1-4 d-old cohorts, to highly heterogenous (b) 1-17-d-old cohorts, all unmated prior to release. Additionally, in reference to mean-cohort age, the timing of the oviposition attractant was either provided to cages (c) initially or (d) delayed until 1-d after maximum mating was observed; in a fully factorial design. Results indicate that fertile egg production can be dramatically increased by using homogenous populations of young-only flies by contributing more eggs (54.0 g total for young-only over 7 d compared to 7.7 over 7 d for mixed; 6.9-fold increase) that hatch more often (2.038-fold increase), an effect that was largely influenced by the behaviour of young females to engage in less risk-prone behaviour, whereas old females haphazardly lay eggs outside of the trap. Additionally, introducing an attractant until after peak mating may reduce conflicts of interest between females that must decide to either acquire sperm via mating or lay eggs, thus contributing to the observed increase in egg weight (30.7 g total for delay over 7 d compared to 17.9 g over 7 d for initial; 1.7-fold increase) and hatch rate (1.3-fold increase), but only in young-only populations. Critically, these results were demonstrated at a density of 100M:100F, and so need to be validated at scale prior to being adopted, and then modelled to see where economic trade-offs occur.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Artificial plant perches reveal sex-specific behaviour in the black soldier fly, *Hermetia illucens*

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Many companies that mass-rear the black soldier fly, *Hemetia illucens*, use perches within cages to create a more natural breeding environment or as a means to provide additional surface area relative to cage volume for flies to rest upon; however, the effect of such on fitness remains untested, nor are the economic-trade-offs of their inclusion. An indoor, laboratory-scale experiment tested the effect of providing perches to black soldier fly breeding cages. Perches were constructed from artificial leaves affixed to wooden dowels inserted into foam blocks, placed in the centre of cage floors. Four treatments had adaxial (top) leaf surface area ranging from 0.00 m^2 (CTRL), 0.04 m^2 (LOW), 0.26 (MED), and 0.34 (HIGH) m^2 per 0.938 m^3-volume cage. Each cage was supplied with a population of 100 male and 100 female flies, with females marked with acrylic paint. Statistical models indicate that the density of perches relative to cage volume had a NULL effect ($P > 0.05$) on (a) the number of clutches, (b) mean clutch weight, and (c) hatch rate. An analytical model presented will project how perches can be utilized to increase the density of flies in cages without negative effect at industrial-scale. Interestingly, a time-series analysis on sex-specific perch utilization revealed that females were (1) more likely to utilize perches than males across all treatments, (2) female-usage increased disproportionately with perch surface area, (3) that usage was extremely female-biased in the morning, (4) that it became less female-skewed throughout the day, and (5) that the pattern repeated each day with a decreasing trend throughout the week. This pattern could be explained by the casual observation that male flies were primarily engaged in aerial contests when artificial lamps were illuminated during early hours, but gradually would leave mating contests to rest, and is in keeping with prior knowledge of how black soldier flies lek in the wild.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Volatolomics reveals behavioural changes of honeybees in response to its exposure to the insecticide Fipronil

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Determining the biological mechanisms associated with a disturbance is the key to study insect's response to a given stress like an exposure to insecticide. In honeybees, these mechanisms may be induced or regulated by Volatile Organic Compounds (VOCs) that may be measured by Volatolomics. Experimental design was performed using emerging honeybees *Apis mellifera* in the laboratory in pain-type cages. Volatolomics analysis were performed on cuticle adhered tissues (abdomen without digestive tract) after 14 and 21 days of chronic exposure to 0.5 and 1 μg/L of fipronil, corresponding to an exposure at sublethal doses. VOCs analysis was processed using a HS-SPME/GC-MS method. A total of 281 features were extracted and tentatively identified., the abundance of 12 VOCs of the volatolome of bees were shown to be changed in response to fipronil chronic exposure. Positive matches with existing database reveal that one of these markers could possibly act on the GABA receptors activity which is known to be a potential fipronil target. Others (pheromones, repellent agent and compounds related to Nasonov gland) could be associated to semiochemical activities leading to a potential impact on bee behaviour.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Standardisation in insect trials: effects of different rearing scales and relative humidity on *Tenebrio molitor*

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Given the relative youth of research in insects as food and feed, experiment protocols often differ and comparison is difficult. This experiment aims at comparing the effects of three rearing scales at two relative humidity (RH) conditions (60% and 70%) on the growth of *Tenebrio molitor* (TM) larvae. The sizes of the experimental unit were: small (13*13*5.5 cm boxes), medium (30*20*11 cm) and large (60*40*10 cm), with six treatments in total: (1) Small scale at 60% RH (S60), (2) Medium scale at 60% RH (M60), (3) Large scale at 60% RH (L60), (4) Small scale at 70% RH (S70), (5) Medium scale at 70% RH (M70), (6) Large scale at 70% RH (L70). Each treatment was replicated 5 times. All the other parameters were kept constant: genetic origin of the larvae, feed (wheat bran), feeding rate, areal density (2.5 larvae/cm^2), volumetric density (1 larva/cm^3) and water source quantity (agar). Trial started with 3 weeks old larvae and sampling occurred every 7 days until 5% of pupae was observed in a replicate. Data were analysed using SPSS software. The different scales did not have a significant effect on the survival of the larvae and the development time at either of the RH tested ($P > 0.05$). The FCR was higher in M70 compared to S60 ($P < 0.001$). The SGR was comparable among the treatments, with the only exception of M70 displaying a higher value when compared to S60 ($P < 0.05$). Regarding larval weight, at week 4, M70 and L70 showed the greatest larval weights ($P < 0.001$). At week 6 and 7, M70 displayed the heaviest larvae ($P < 0.001$). At the end of the trial, larval weight was comparable for M70, L70, S70 and M60 ($P > 0.05$). In conclusion, the size of the experimental unit at the two RH conditions tested has an effect on the growth parameters of the larvae and it should be taken in consideration when planning experimental protocols and comparing experiments. The trial is supported by Agritech National Research Centre (PNRR CN 00000022).

INSECT BREEDING, GENETICS AND BEHAVIOUR

Evaluation of adult behaviour of the black soldier fly under natural lighting condition

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The aim of this research was to increase knowledge on the biology of black soldier fly adults (BSFA) by building a database of morphometric, climatic, behavioural, and demographic data on BSFA. The second aim is to design a valid and easily replicable experimental protocol for BSFA behaviour. A cage (60 × 60 × 90 cm) was placed in a climatic room (27 ± 1 °C, 60% RH) with 1000 g pupae to obtain adults. 100 BSFA (sex ratio 1:1) were placed in each of 3 trail cages (30 × 30 × 40 cm) where the behaviour observation took place under sunlight. The adults were marked on the thorax with different acrylic markers, weighed and photographed to record all the morphometric parameters. Three trials were set up to evaluate the following behaviour: mating, missed mating, fights, oviposition and missed oviposition. In the first trial only adult males (M) were marked, females (F) in the second one and both M and F were marked in the mix (F+M). Morphometric results reported a length of 15.8 ± 1.07 mm and a weight of 80.1 ± 16.58 mg in M, while in F, 15.1 ± 1.84 mm and 68.8 ± 21.79 mg, respectively. F+M reported a length of 16.4 ± 9.05 mm and weight of 97.5 ± 16.62 mg for F, while 15.0 ± 0.81 mm and 75.3 ± 14.76 mg for M. Behaviour results showed the peak number of mating and fights occurred at 9.30 (6,930.9 l×, 26.3 °C and of 59.7% RH) in the M trials. The maximum number of missed mating occurred at 11.30, (12,736.7 l×, 30.4 °C and 49.5% RH). In the F trial, the peak of the number of mating and missed mating occurred at 10.30 (13,367.5 l×, 30.6 °C and 49.9% RH). The peak of oviposition and missed one occurred at 14:00 (7,388 l×, 31.9 °C and 46.9% RH). The matrix of correlation between the environmental and behavioural variables showed as: mating and missed mating are strongly and positively correlated to each other (0.56); oviposition number and missed oviposition are positively but weakly correlated to each other (0.32); temperature and humidity are highly correlated to each other but inversely correlated with behavioural variables (−0.98); the number of mating is strongly and positively correlated with light (0.73); deposition number and light are negatively but weakly correlated to each other (−0.34).

INSECT BREEDING, GENETICS AND BEHAVIOUR

Proof of concept of pedigree-based family selection in *Hermetia illucens*: genetic parameters and response to selection

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Long-term succeed of the insects as food and feed industry still requires developments in many fields, from value chains to industrial processes, from zootechnical levers to selective breeding practices. With a 45 days life cycle in *Hermetia illucens*, the prospect of fast genetic gains is thrilling. However, several challenges need to be overcome first: genetic parameters for traits of interest are often unknown and the phenotyping tools to measure them are limited, BSF husbandry practices are still in their infancy and the impact of the environment on phenotypes is high. Pedigree-based family selection directly addresses the estimation of genetic parameters for multiple traits while factoring in environmental variations. Additionally, traits can be measured on parents or their siblings. Here, we build a pedigree-based family selection system, where mating occurs at a family level. Variance components and estimated breeding values (EBVs) of traits of interest were estimated using respectively the REML (restricted maximum likelihood) and BLUP (best linear unbiased prediction) statistical models. Briefly, egg clutches were used as full-sibling families, which were crossed in groups of 20 pairs. Production traits (final wet weight for the first growth phase; of the second growth phase ad libitum, LW2 and in restriction, LW2R) and reproduction traits (pupal perimeter, female weight at emergence, thorax length and ovocytes number) were evaluated on 15 to 24 families, on 10-30 individuals per family for 3 to 12 generations. The heritability for LW2 was relatively low (0.13 ± 0.04), while LW2R was moderate (0.58 ± 0.03). Direct selection on LW2 resulted in an increase of EBVs. Phenotypic gains were assessed after 5 generations against an unselected population and showed a gain of 11% for LW2 and a GxE effect. This proof of concept clearly demonstrates that a pedigree-based family model can be of practical use as a breeding scheme in BSF but needs to be further refined, to control consanguinity and the complexity of the mating structure, and to ensure scalability in an industrial setting.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Unlocking the potential of selected breeding in black soldier fly farms: a SNP panel approach

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The maintenance of healthy broodstock is a long-standing concern in black soldier fly farming. However, to date, the application of molecular approaches to the management of BSF commercial farming has been lacking. This study initiates research into the utility of BSF molecular tools by employing molecular markers to quantify the impact of artificial selection on BSF performance and diversity. In a multi-generational and multi-population study, BSFL growth rate, BSF emergence, and egg production were monitored and used for selective breeding. Using a low density panel with 400 bi-allelic single nucleotide polymorphisms (SNPs) across the *Hermetia illucens* genome, 672 individual BSFL were genotyped and population gene diversity, heterozygosity, relatedness, and inbreeding coefficients determined. Mean number of alleles over all loci were 1.81-1.98, while gene diversity ranged from 0.254-0.357. Populations reared at the commercial scale revealed high observed heterozygosity, which were consistently lower than the expected heterozygosity, indicating some level of inbreeding in the population. However, relatedness distribution of pairwise comparisons indicate 99.8% of individuals do not show levels inbreeding depression ($r > 0.25$). These levels are consistent with yearly monitoring and did not change within a 6-month monitoring period. The results suggest that the directional selection of BSF populations towards trait homogeneity can be accomplished without compromising genetic vigor. Further, a low density SNP panel can be used to recurrently sample and monitor the genetic diversity of a commercial population and assist in making decisions related to new population introductions, scaling up production, and crossbreeding. We highlight the necessity for implementing genetic and genomic tools in BSF breeding programs to drive trait improvement in BSF rearing while maximizing genetic diversity for BSF production longevity.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Genetic strategies for black soldier fly (BSF) production

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The rapid generation interval and prolific reproduction of black soldier flies (BSF) suggest significant potential for selective breeding. The aims of our study were to estimate variance components of commercial traits at group and individual level under a breeding programme scheme; to evaluate different breeding strategies and optimise them to achieve a competitive genetic gain that can be achieved practically and sustainably for the long term; and to assess the genetic differences between a selectively bred population and a production line. Phenotypic data on larval body mass was collected over a year at group and individual levels and analysed using univariate genetic models. A breeding program was simulated for body mass over six years, with between and within-family selection without sexing. Genetic differences between selectively bred and production populations were assessed using pool sequencing. The ratio of group variance to phenotypic variance for body mass increased from 0.15 to 0.42 when collected at the group versus individual level. Simulation results demonstrated a selection response over time, with higher selection intensity within families leading to increased female proportion. Pool sequencing identified key genes associated with production traits, with a significant genetic variance observed between populations. Differences in variance ratios between group and individual levels highlight the importance of within-family variance in accelerating trait improvement. Simulation outcomes suggest that response to selection can be optimized through varied phenotyping and selection proportions. High selection intensity on body mass influences gender distribution in cages post-selection resulting in population crashing over time. The study indicates the feasibility of a standard breeding program for BSF, with the potential for generating selection response over time. Optimization strategies include individual phenotyping, varied selection proportions within families, while caution is warranted against excessive selection intensity within families for sustainable selective breeding.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Effect of high temperature stress on black soldier fly pupation and emergence

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Research on black soldier fly (BSF) rearing conditions, from larvae to pupae, is commonly conducted within laboratory settings with controlled environments and constant temperature. However, in tropical mass-rearing facilities, rearing conditions without significant environmental control features can be advantageous in terms of lower costs and lower energy requirements. The major downside of these rearing systems is that the temperature fluctuation depends on the weather and the impact of this on the prepupae is not well known. This study examined the impact of thermal variation and thermal tolerance on BSF pupation and emergence. Initially, data was collected from a mesh-sided structure in peninsular Malaysia (latitude of 1.4927°N) to understand the impact of different thermal regimes on BSF prepupae. The initial observational data indicated that temperatures above 37 °C were correlated with negative reproductive outcomes. To understand the impact of this temperature threshold limit, the treatment prepupae were exposed to thermal shock for 4 hours at 38 °C at varied prepupae holding days. Meanwhile, the Control prepupae were left inside the modified outdoor cage for 10 days with daily peak temperature below 37 °C. Results showed that thermal shocks significantly affected prepupal development time, and prolonging the days with thermal shocks extended the prepupae recovering time. The thermal shock did not significantly affect the adult emergence rate for one day of exposure, however, there was up to a 10% decrease for prepupae exposed to daily thermal shocks for 5 consecutive days. This research adds to our understanding of thermal tolerance in prepupal BSFL and its implications for mass production systems.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Genetic parameters for larval body weight, development time and protein content in *Hermetia illucens*

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The insect-based conversion of low-grade organic waste streams into high-value protein for animal feed plays an essential role in circular food production efficiency. However, information on genetic parameters crucial for insect production, such as larval body weight, development time, and protein content, is predominantly lacking. This study aimed to estimate the heritability of these traits for an Indonesian strain of *Hermetia illucens*. A full-sib design was implemented in a practically feasible manner to record multiple traits within families. Egg clutches from 50 families were divided into two rearing groups, housed separately to distinguish the common environmental effect from the family effect. To obtain records for larval body weight and development time, 20 larvae per family environment group were individually measured and then housed until emergence to record development time and the sex of each individual. Protein content was measured as a joint sample of 50 larvae, repeated three times per family environment group, resulting in six protein records per family. Genetic parameters were estimated using a linear mixed model. The heritability of larval body weight was estimated at 0.20 (0.11). No family variation was observed for development time. For protein content, the heritability was 0.94 (0.18), acknowledging the limited precision due to only 300 records; nonetheless, notable variation between families was evident. The common environmental effect was 0.25 (0.05), 0.09 (0.02), and 0.27 (0.07) for larval body weight, development time, and protein composition, respectively. The common environment persisted only until the individual weighing moment at the larval stage and protein sampling; thereafter, the insects were housed individually, resulting in a limited common environment effect for development time. These results suggest the feasibility of selecting for body weight, and indicate potential for protein content improvement in insect populations.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Establishment of platforms for genetic manipulation for black soldier fly *Hermetia illucens*

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The black soldier fly, *Hermetia illucens*, is a valuable insect resource due to its ability to efficiently digest organic waste and convert it into valuable proteins and fertilizers. Genetic manipulation platforms can be instrumental in breeding the black soldier fly to meet various needs. In 2020, we successfully edited the genome of target genes and announced the genome sequences of the black soldier fly. To facilitate genetic manipulation of this economically significant insect, we have developed the first transgenic methods. We identified the ubiquitous actin5c promoter (Hiactib5C-p3k) and three endogenous U6 promoters (HiU6:1, HiU6:2, and HiU6:3). The Hiactin5C promoter was utilized to drive the expression of a highly active variant of the piggyBac transposase, resulting in a transformation rate up to six times higher than that of the wild-type transposase. Additionally, we assessed the suitability of the three HiU6 promoters in this transgenic system. HiU6:1P and HiU6:2P emerged as the most promising candidates for driving the expression of guide RNAs in CRISPR/Cas9 or other gene editing systems. Overall, our findings offer valuable genetic engineering tools for fundamental research and genetic manipulation of the black soldier fly.
Keyword: Black soldier fly, transgenesis, hyPBase, actin5C promoter, U6 promoter.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Genomic diversity in *Hermetia illucens* (BSF)

B. Gradus

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Insect farming, specifically the bioconversion of organic material into animal feed, is increasingly making use of the black soldier fly (BSF). However, in comparison to other farm animals, the genetic resources of farmed insects remain poorly characterized. NRGene is a leading molecular genomics company where we utilize our pipelines to develop and apply modern molecular breeding tools for the BSF industry. As part of an Israeli BSF consortium, we generated the first two BSF high-quality PHASED (separate reconstruction of the sequences corresponding to the two copies of each chromosome) chromosome-scale genome assembly (male and female). These genomes were then used for downstream analyses for population genetic characterization and diversity. To accomplish this, we leveraged our proprietary DeNovoMAGIC™ pipeline, which combines Pacific Bioscience long reads with Illumina short reads sequencing to serve as input for the assembly. BUSCO benchmark resulted in a score of 96.04% for BSF IL_Male and 96.13% for BSF_IL_Female. While investigating the assembled genomes, we noticed that the coverage of the contigs of chromosome 7 (the suspected X chromosome) was 50% as compared to the rest of the chromosomes. This finding supports the XY dependent sex determination in BSF. Further comparison of the two phased genomes with the un-phased (collapsed) iHerIll genome, identified unmapped scaffolds unique to the BSF_IL_Male assembly, suggesting that these scaffolds may belong to the previously uncharacterized male Y chromosome. The BSF_IL_Male assembly served as a reference genome for our diversity analyses and population genetics comparisons. Whole genome sequences of more than 16 populations (5 male and 5 females per population) were produced for this purpose. The results illustrated the high diversity that exists between populations from around the world. We also found that the Y-related scaffolds segregate in the population and exist only in males, validating our hypothesis that these scaffolds belong to the Y chromosome. Next, we generated a genetic SNP panel that harbors this diversity and male-female identification SNPS. Using our genomic resources, we encountered the interesting observation of high chromosome level diversity of the X chromosome in different populations.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Study into *Hermetia illucens* behaviour in large artificial mating chambers and possible optimizations

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InsectoCycle has been researching black soldier fly (BSF) reproduction for four years, focusing on adult fly behaviour as a pivotal factor in efficient mating. Despite advancements in entomological studies on fly mating behaviour, significant gaps remain. Our team has delved into understanding the nuances of the behaviour, rheotaxis and climatic preferences of adult flies at various stages. We repeated density observations in two-choice experiments to understand the rheotaxis of the flies. The distribution of approximately 300,000 flies over the two choices was visually estimated over two weeks, after which we switched the airflow for another two weeks. This experiment was repeated twice. To analyse the climatic preference of the adults, we took 3 samples of 30 flies per location in the cage and scored their age, mating state and gender. This experiment was repeated twice. From these results we tried to extrapolate what locations the different genders and life stages prefer and how they are spread throughout our cage. This led to modifications in our mating chambers in which we direct the airflow to come from the top and create two different zones. One with cleaner air for mating and one for stimulating egg laying by providing an attractant. This presentation will go into the rheotaxis experiments, detail the method of scoring flies their age and mating status, offer the conclusions of our observation and possible ways to optimise fly behaviour in artificial mating environments using airflow and extra wall area.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Influence of water supply on reproductive performance in adult black soldier flies in industrial scale production

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The black soldier fly (BSF), *Hermetia illucens*, has emerged as a key player in industrial waste management and sustainable protein production. In large-scale operations, understanding the impact of water supply on the reproductive performance of adult BSF is essential for optimizing efficiency. This study aimed to assess how water availability influences key reproductive parameters in adult BSF, focusing on aspects relevant to industrial-scale production. A preliminary experiment was conducted with two distinct water supply types tested (tap water and sugar water), in the same as the abiotic conditions of industrial-scale production conditions). Results demonstrated the effect of water supply centred on the extension of adult BSF lifespan by 2 days. Adequate water availability could positively influence a robust and productive BSF colony. Conversely, water scarcity led to a notable decline which could impact overall efficiency and output. The water supply experiment was conducted on an industrial-scale adult fly cage for egg/neonate production. The abiotic conditions in both experiments were a mesh-sided building exposed to ambient weather conditions in Johor, Malaysia. The results demonstrate no significant difference in the neonate production for four batches tested even with the expectation of lifespan extension as in the preliminary experiment. However, qualitative observations of fly behaviour indicated they were attracted to the water supply area. As other researchers have shown, our study indicated that water availability has a variable impact on BSF lifespan and performance. Future research should consider the impact of water when environmental conditions are variable and include heat stress or low humidity conditions.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Reproductive system development and assessment in *Hermetia illucens* females (Diptera: Stratiomyidae)

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The black soldier fly (BSF) has the potential to play a significant role in the production of protein, oil, and insect fertilizer in the near future. Due to their ability to convert various waste materials into biomass, BSF production has been scaled and industrialized globally. One of the most critical factors for continuous bioconversion activities is the availability of eggs. While information is available on the morphology of the female reproductive system, the assessment of ovarian morphological dynamics and its interactions with mating status has received little attention. Novel approaches are needed for evaluating the sexual maturation of BSF. The objective of research was to characterize the development of the female reproductive system and to evaluate novel approaches for assessing reproductive status in BSF. The following tasks were performed: (1) Classify the ovarian maturation process into developmental stages; (2) Examine traits of female reproductive system and insect sizes for each developmental stage; (3) Utilize these parameters to assess the female reproductive status. Insects were reared under controlled conditions in industrial BSF rearing facility. Females were dissected at different age groups. A total of 200 flies were examined. The following ovarian morphometric traits were measured: ovary length (OL), ovary width (OW), ovary index (OI = OL*OW). Additionally, wings length (WL) and thorax length (TL) were measured as indicators of female size. All quantitative data were analyzed using the IBM SPSS Statistics software. Ovarian development in BSF goes through five stages. The corresponding reproductive status of females is described for each developmental stage. OI and OL proved to be reliable indicators ($P < 0.001$) for assessing the level of sexual maturation of BSF. WL and TL are not the most useful measurements for evaluating reproductive status in BSF, although WL can partially enhance evaluations using OL and OI ($P < 0.001$). These findings suggest that the studied approach can be effectively employed during the breeding process for precise reproduction and maintenance of BSF adult colonies.

INSECT BREEDING, GENETICS AND BEHAVIOUR

On a quest for superior strains: a progress report on Haid group's black soldier fly selective breeding program

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The black soldier fly (BSF) is highly effective in upcycling organic waste into valuable feed ingredients approved for animal diets by the US and EU. The increasing demand for high-quality protein and organic waste reduction has created a need for greater quantity and quality of BSF products. However, selective breeding in BSF has been sparsely implemented. Haid Group as the world's largest aquafeed company, has a strong demand for alternative protein resources to replace fish meal and has made promising strides in a BSF selective breeding program aimed at improving production traits. We strongly encourage colleagues to initiate their own breeding programs and consider collaborating to develop superior strains of BSF, thereby propelling the industry to new heights. Multiple strains were established by selecting individual BSF based on different production traits, obtained from a BSF production facility operational for over four years. Each strain underwent continuous inbreeding for more than one year. The target traits of all strains were consistently monitored throughout each generation, unless no viable offspring were produced. The rearing environment was carefully maintained at a temperature of 30 °C and a relative humidity of 70%. Several selected strains were subjected to crossbreeding experiments to evaluate the effect of heterosis. Significant improvements were observed across all selective breeding experiments, with different traits displaying varying degrees of improvement and stability over generations. Positive heterosis was consistently observed in the crossbreeding experiments, persisting for multiple generations. To ensure a successful breeding program, it is crucial to address three key elements: genetic diversity, inbreeding depression, and sex separation. We strongly recommend forming a breeding alliance to collectively tackle these issues as a team. The BSF industry stands to benefit greatly from a selective breeding program that creates strains with improved production traits. Given the short life cycle of BSF, positive results can be achieved within a relatively short period of time.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Parental assignment in black soldier fly (*Hermetia illucens*, Linnaeus, 1758) using SNPs genetic markers

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In the fast-growing industry of black soldier fly (BSF), developing high-performance strains will allow for more effective production. Unfortunately, polyandry makes classical methods of genetic selection nearly impossible to implement in BSF. Our project aims to develop a parental assignment tool based on SNP-type genetic markers. The first step was to design a method of production that would allow control of the genealogy of each specimen for multiple production cycles. For each generation (n = 5), 200 adults were maintained in darkness from emergence to mating, which was initiated by placing the cages under light. A total of 24 families were created at two experimental sites (university (A) and industry (B)). The first four mating couples of each family were isolated. Collected eggs were suspended above Gainesville diet ({[cursief]}ad libitum, {[/cursief]}70% RH). At 5-day-old, only the larvae (n = 200) of one mating pair of each family were kept for the next generation. For each family and each generation, the mating pair and two larvae were preserved at -20 °C for DNA extraction and GBS sequencing (n = 480). After SNP calling and quality control, high-quality SNP markers will be retained. Likelihood-based simulations will then be conducted to identify the smallest subset of markers that can be used for accurate parental assignment. The phenotypic data of the first 4 generations (G) were analysed for trends between generations. Upward trends were identified for the pre-oviposition period at the B site (G1: 2.96 ± 0.80 days; G4: 3.44 ± 0.96 days) and the pre-hatching period at both sites (G1: 2.4 ± 0.8 days; G4: 3.1 ± 0.8 days). No overall trend was observed for the mean weight of 5- (A: 79.2 ± 25.1 mg; B: 53.9 ± 21.8 mg) and 10-day-old larvae (A: 235.0 ± 29.8 mg; B: 212.2 ± 26.9 mg) at either site. These preliminary results indicate that inbreeding might influence the reproduction cycle without affecting the larval growth performance.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Quality in insect processing: building the foundation of insect product grading

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Insect farming has grown significantly around the world over the past decade, resulting in wider availability of insect products such as whole dried, purees and meals. With the industry still in its infancy, there is a great range of methods and processes applied to the creation of insect products, resulting in a broad range of product specifications and qualities. With insect product costs remaining relatively high compared to mainstream nutrient sources such as soy and fishmeal, one of the principal markets for insect product commercialisation remains pet food. Here, insect products are often marketed not just as sustainable, but premium functional products. However, not all insects are made alike. Due to the sheer novelty of insect ingredients, there is a general lack of understanding amongst nutrition professionals on what a good insect product might look like, contrary to such knowledge being commonplace for existing key ingredients. This, coupled with sometimes a poor understanding of the subject area in new insect producers, can result in bad customer experiences that dissuade future investigation or use of insect ingredients by formulators, especially in quality-sensitive segments such as premium and ultra premium pet food. Here we focus on *Hermetia illucens* products as an example to illustrate the range, and implications, of varying product qualities across both wet and dry products. A tentative grading system is proposed to assist insect producers and insect product users in ensuring best product-market fit, and better understanding the premium (or lack of it) that their product might be able to fetch based on its quality properties.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Hatching time and frequency of neonates in black soldier fly

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Understanding the hatching time and frequency of neonates in the black soldier fly (BSF) is vital for effective production planning in BSF farming and expanding our knowledge of the species' biology. However, there is currently a lack of accessible data on the hatching characteristics of BSF eggs. This study aimed to address this gap by investigating the hatching time and frequency of BSF neonates, offering valuable insights for production planning in BSF farming and enhancing our understanding of fly biology. The eggs used in the experiment were obtained from a semi-controlled production setup at Entofood Sdn Bhd. The lighting in the breeding cage was set to 13 hours of light (6 am to 8 pm) and 11 hours of darkness. The incubation time was counted from the moment the eggs were collected from the cage and transported directly to the incubator. To generate comprehensive data on hatching frequency, the test was repeated eight time. Each replicate exclusively sourced eggs from a single harvested batch, following a standard daily egg harvest procedure. Once the neonates hatched, they were systematically collected, weighed, and recorded every hour from the first neonate until the emergence of the last one. The incubation conditions were maintained at a stable temperature of 30 °C with 80% humidity. The findings revealed that after three days of incubation (72 hours from the moment the eggs were laid by the female in the cage), over 94% of the eggs had hatched, with the remaining <6% hatching within the following 10 hours. This suggests that an 82-hour incubation period is necessary to ensure the hatching of all fertilized eggs. The egg-to-neonate hatching rate was 100%. The outcome of this study has practical implications for BSF farming. By uncovering crucial information on hatching time and frequency, this research enables effective production planning, ensuring a consistent and reliable supply of larvae for applications such as animal feed and waste management. Additionally, this study contributes to the knowledge of BSF biology by providing insights into their life cycle and reproductive patterns.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Producer's great and small – We need them all! A breeder's perspective on black soldier fly production

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From our vantage point as a leading genetics company specializing in developing and distributing black soldier fly breeds, this talk will provide a nuanced perspective on industry trends. We've observed a marked uptick in demand for black soldier fly (BSF) egg and juvenile larvae production, as operators gravitate away from specialization in either egg or larvae production. We delve into the dynamic landscape of insect farming, fuelled by the recent influx of insect farming equipment suppliers. This development has democratized access to insect farming, attracting a diverse array of participants ranging from retailers to food waste processors and enterprising farmers embedded within the agri-food chain. Combining a mix of industry intelligence, production experience, and genetic knowledge, this talk will cover: (1) What producers will need to achieve in terms of environment and capability to unlock the full potential of genetics. (2) Transitions in egg and 5-DOL supply dynamics, which will transition towards a hybrid model encompassing both on-site production and co-located egg production facilities. Over time, we anticipate a gradual decline in on-site egg production as producers pivot towards larvae production. (3) A look to the future in terms of what genetics can offer - development of specialized lines tailored to different geographies/climates, diverse feedstocks and to exhibit specific traits/features (e.g. egg production, larval growth). We will also provide our perspective on what future production requirements will look like – a network of facilities that are facilitated by genetics developers and distributors in conjunction with egg producers, comprising both centralized and co-located operations. Collaboration emerges as a cornerstone of industry success, critical for addressing pressing environmental imperatives. Covering the above topics, this talk promises to offer a comprehensive understanding of the evolving landscape of insect farming. Our insights aim to equip attendees with actionable strategies for success in the burgeoning insect farming sector – because regardless of whether producers are big or small, to overcome our global challenges, we're going to need them all.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Cage design for black soldier fly mass production: exploring biomimicry in love cage design

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Optimizing mass production of black soldier fly (BSF) needs proper design of love-cage. Understanding the lekking behaviour of BSF adults is crucial for eggs production, as adults require surface areas for fighting, resting, and reproducing. This study uses a shallow biomimicry design approach, which involves replicating the spatial arrangement of natural habitats in the design of artificial habitats. 3 love cages per treatment (1 m^3) were placed in a climate chamber (27 ± 1 °C, 60 ± 5% RH) using LED lights for BSF. Pupae were continuously loaded to obtain a density of about 1,600 flies (sex ratio: 1.45). The first trial evaluated the variation of the different surface area/volume values by adding additional surfaces: A (control: 3.33 m^{-1}), B (3.99 m^{-1}), C (5.11 m^{-1}), and D (7.17 m^{-1}) treatments. The treatment that showed the highest egg production was selected for the colour trial.Three colours were considered: green, white, and mixed. Colour is a crucial factor in determining the wavelengths that the adults perceive, stimulating mating. In the colour trial, the control cage was A. The wooden eggs traps were collected and weighed 3 times a week. Both trials lasted one month. The Anova test was used to analyse normally distributed data, while the Kruskal-Wallis test was used when the normality of the data was not verified. Results from the first trial showed high average egg production using B (18.6 ± 4.42 g), followed by C (15.0 ± 3.10 g), D (11.3 ± 3.62 g), and A (9.23 ± 3.14 g) treatments. Statistically significant differences were observed only between the control vs B treatment. White reported the highest eggs production (19.4 ± 4.85 g), followed by green (15.02 ± 2.06 g), mixed (10.4 ± 1.54 g), and control (9.1 ± 3.58 g). Significant differences were noted between the control vs white, control vs green, white vs mixed, and green vs mixed. The adoption of a shallow biomimicry approach in love-cage design demonstrated a notable increase in BSF egg production. Both the geometry and the colour in the love cages are of considerable importance and need further exploration. Future trials will extend to larger industrial love cages (24 m^3), contributing to scalable and efficient BSF mass production systems.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Sex-specific plasticity across various black soldier fly lines suggests trade-offs in adult reproduction and longevity

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Black soldier fly larvae (BSFL) are well-documented for their ability to consume a wide range of organic matter. They exhibit remarkable plasticity in their juvenile development, largely attributed to their ability to adapt in nutritionally diverse substrates. BSF research worldwide have highlighted substantial variation in BSFL growth and development, possibly attributed to the different BSF populations used across various research institutes. However, there is limited understanding of how the substrate influences BSF adult phenotype, including its reproductive trait and adult longevity. This study addresses the gap by using eight different genetic lines of BSF and reared on two divergent substrates (homogenous chicken feed and heterogenous food waste). The research focuses on tracking the larval growth rate, adult morphological and reproductive traits, and their overall life history. Although there was a hysteresis in the juvenile development (larval and prepupal stage) of BSF when they were acclimated to new environments, different genotypes generated divergently shifting trends in adult phenotypes (morphological and reproductive traits). Notably, the study highlights the influence the substrate has on the reproductive traits across genetic lines, especially the variations in female ovaries and accessory glands, as well as male accessory glands. Testes size seemed to be instead largely influenced on the genetic line. The findings from this study underscore the relationship between diet, genetic factors, and reproductive investment in BSF, providing insights into future research and industrial applications.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Genetic diversity and improvement of the black soldier fly

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To improve food security, sustainable agricultural practices are needed due to threats posed by climate change and population growth. Insect livestock, specifically the black soldier fly, is a promising organism for circular food production by feeding on organic waste. However, knowledge of the fly's evolution, genetics, and potential for genetic improvement is lacking. We have been studying the domestication and genomics of *H. illucens*, revealing evidence of considerable genetic diversity around the globe, and for selective sweeps in the genome associated with domestication. Domestication has likely occurred independently multiple times but is associated withe genetic changes in the same regions of the genome. We have used experimental evolution to select for increased pupal size, with correlated improvements in many industrially important traits. We have also developed CRISPR/Cas9 approaches for genetic modification that hold considerable promise for the future. We are also using high throughput microfluidic techniques to screen the genome of BSF for enzymes with economically relevant catalytic activity. This work lays the foundation for *H. illucens* as an important global agricultural organism.

INSECT BREEDING, GENETICS AND BEHAVIOUR

Innovative phenotyping systems to advance selective breeding in black soldier fly: status from the FlyGene project

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Despite the promising potential of selective breeding in insects, its application to enhance the quantity and quality of black soldier fly (BSF) products has been largely overlooked. The challenge of phenotyping various traits has impeded selective breeding implementation in BSF, primarily due to the insects' small size and the high-density production system. Supported by the Danish Ministry of Foreign Affairs, the FlyGene project aims to address these challenges through research-led knowledge generation and capacity building. A key objective is the development of innovative phenotyping systems utilizing computer vision and multi-spectral imaging approaches to measure traits such as larval weight, sex, protein, and fat content. In an initial study employing deep learning techniques, correlation coefficient of up to 0.91 between predicted and measured larval weight from individual larvae images was achieved. Furthermore, deep learning models trained on image data exhibited a prediction accuracy of 74% for larval sex. Additionally, promising results were obtained for predicting lipid and protein contents using multi-spectral image data analysis. These findings highlight the potential of computer vision and multispectral imaging approaches to advance selective breeding implementation in BSF and other farmed insects.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Enterococcus regulates amino acid metabolism in the edible insect *Clanis bilineata tsingtauica*

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Effective utilization of edible insects can help alleviate the global food security crisis. The soybean hawkmoth, *Clanis bilineata tsingtauica*, is an edible insect with high nutritional, medicinal and economic value. It has become a characteristic agricultural industry in China. This study investigates the regulation of nutrient metabolism in diapause larvae of *C. bilineata tsingstauica* through nutrient physiology assays, gut microbiome and non-target metabolomics analysis. It was found that although diapause larvae had entered a state of developmental arrest, they still maintained a stable weight and balanced nutrition. However, the content of certain amino acids and fatty acids varied with diapause duration. For example, an increase in diapause time led to higher levels of leucine, phenylalanine and arginine in larvae. Analysis of 16S rRNA sequencing revealed that the most prevalent taxon was the genus Enterococcus. Metagenomics analysis identified *Enterococcus casseliflavus* as the dominant species. The gut microbiome analysis showed that the dominant bacterium was primarily involved in carbohydrate, amino acid and energy metabolism. The non-target metabolomics results indicated that the top 20 enriched KEGG pathways were mainly focused on amino acid metabolism, global and overview maps, and digestive system. Three differential metabolites were identified: N-acetyl-d-glucosamine, trehalose and D-glutamine. On diapause day 28, the content of D-glutamine involved in arginine biosynthesis pathways was significantly higher compared to day 0 and 14. It is speculated that *E. casseliflavus* may increase the arginine content and alter the metabolite D-glutamine of larvae by regulating arginine metabolic pathways. This study provides insights into the gut microbiome of *C. bilineata tsingstauica* at the species level and offers preliminary information on the regulatory mechanism of Enterococcus that contributes to the nutritional metabolism of insect hosts.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

New insight on mass rearing black soldier fly state of health revealed by DNA-based molecular tools

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Despite black soldier fly (BSF) being known for its robustness to insect diseases, mass rearing industries are reporting increasing numbers of BSF mortality events. Veolia Bioconversion Malaysia is an industrial plant producing BSF meal from GMP+ certified feedstocks. In 2023, an event of mortality was observed in the breeding centre (BC). Samples were collected, during this event, from the BC at different stages (egg, neonate, larvae) and state of health (live and dead) but also from the feedstocks and grow-out area, to investigate if DNA molecular tools could highlight a microbial indicator of BSF mortality. DNA was extracted using an adapted commercial kit and sequencing was performed on a Illumina MiSeq platform using QIAseq Panels for simultaneous V3-V4 and ITS regions amplification. Data were processed using FROGS pipeline and statistically analysed using Phyloseq R package. For fungal sequencing, the main fungal genera retrieved in feedstock samples were *Saccharomyces* and *Candida*, whereas they were underrepresented in the larvae samples, suggesting fungal growth was inhibited in the feeding larvae. Regarding bacterial sequencing, PCoA on Bray Curtis distances revealed that samples statistically grouped according to live/dead status (*P*-value 0.001). Interestingly the genera differentially more represented in live-BSF samples mainly contained well-known probiotic species, such as *Bifidobacterium* and *Limosilactobacillus*. In contrast the genera associated with dead-BSF samples were *Morganella*, *Providencia* and *Paenibacillus*. These genera were also present in live-BSF samples, but their high proportion (up to 80%) in dead-BSF suggests a dysbiosis phenomenon. Indeed, *Paenibacillus thiaminolyticus* has recently been shown to infect and kill BSF larvae, resulting in significant increase in *Dysgonomonas*, *Morganella*, *Myroides* and *Providencia* in the BSF gut (She *et al.*, 2023). The present study provides additional evidence on the importance of monitoring entomopathogenic organisms in mass-rearing industries and supports the need for conducting additional research on this topic.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Exploring the diet-dependent composition of the gut virome in *Hermetia illucens* larvae

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The black soldier fly (BSF) is one of the most mass-reared species for insect biomass production, requiring basic research to optimize BSF production efficiency on various diets. One area of focus is the gut microbiota, as it exerts important functions for animal health and performance. Hence, we should not look at the insect as a separate entity but as a 'holobiont'. This means considering the insect with all its symbionts (e.g. viruses, bacteria, fungi), as the functional biological unit. This increases the organism's complexity and requires the characterisation of the entire BSF microbiota to better understand how this insect interacts with its environment. In this study, we zoomed in on the underexplored gut virome, including eukaryotic and bacterial viruses, using an untargeted viromics approach to study its dynamics due to a diet change. Briefly, whole larvae and gut samples were collected at the end of three rearing cycles (16 day-old larvae) on two diets (chicken feed and artificial swill), processed according to the NetoVIR protocol to purify viral particles and sequenced on the HiSeq2000 Illumina platform. The ViPER bioinformatic pipeline was used for analysis. Thirty-three viral genomic contigs were identified, yet each sample was dominated by only 1-3 viral contigs. Furthermore, there is a clear and significant difference in those dominant contigs between diets. The virome of larvae grown on chicken feed is dominated by a single member of the *caudoviricetes* order (58.45 kb) with proteobacterium as a predicted host. After a diet change to artificial swill, the most abundant virus is an eukaryotic virus of the *Totiviridae* family (7.45 kb), which was recently described to infect BSF (Pienaar et al., 2022). Our findings reveal that diet has a strong impact on the gut virota. Future research will have to demonstrate if and how these alterations are correlated with altered health and growth performance of BSF and how we can exploit these insights in microbiota engineering.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

The role of *Hermetia illucens* larval midgut in the reduction of *Staphylococcus aureus*

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The use of black soldier fly larvae (BSFL), *Hermetia illucens*, for the valorisation of organic waste allows to convert low quality biomasses into proteins, lipids, and chitin, which can be employed in different sectors. The risk of contamination of BSFL by food pathogens that are present in the organic waste used as rearing substrate represents a serious concern and deserves to be considered to guarantee high quality insect-derived products. One of the most common bacterial pathogens associated with the organic waste is *Staphylococcus aureus* and, although the literature provides evidence regarding the ability of BSFL to reduce its concentration in the rearing substrate, little is known about the mechanisms involved. In addition, no accumulation of *S. aureus* in the larvae body occurs and how BSFL counteract this pathogen remains unknown. To investigate the fate of this bacterium, chicken feed was inoculated with 7 log CFU/g of S. aureus and the presence of the bacterium in different BSFL gut regions (i.e. anterior, middle, and posterior midgut, and hindgut) was quantified 3, 24, and 48 hours after its inoculation in the rearing substrate. Interestingly, no colonies were detected in the posterior midgut at each time point, indicating the middle midgut environment as an efficient barrier against *S. aureus*. For an in-depth analysis of the potential players responsible for pathogen reduction, the lumen content of the middle midgut was isolated and the involvement of acidic pH, antimicrobial peptides, and lysozyme were assessed. Our results demonstrate that the joint action of antimicrobial proteins and pH are fundamental to rapidly reduce *S. aureus* load in the gut lumen: the pH likely weakens the pathogen and then antimicrobial proteins kill it. These findings represent a starting platform to understand the fate of other food pathogens during their transit through the BSFL gut, like Salmonella spp. The study was supported by EMBO Short Term Fellowship.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Probiotic potential of bacterial strains from the gut of wild and industrially produced crickets and locust

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Orthoptera are predicted to have a market worth of US $3.5 billion by 2029. Cricket production depends primarily on feed quality, often chicken feed, which drives up costs. A possible solution is using agri-food side streams, but some experiments have shown that these lead to lower insect performance due to high fibre and low protein content (Kuo and Fisher, 2022). This work hypothesizes that previous experimental failures to integrate plant-fiber-rich side streams into orthopteran diets is due to the fact that the gut microbiota that degrades these plant polymers is missing or reduced by continuous laboratory rearing. Here, we analyze the gut microbiome and test the probiotic effect of plant polymer degrading, nitrogen waste recycling, and nitrogen-fixing bacteria isolated from the gut system of wild and farmed *Gryllus bimaculatus*and *Locusta migratoria*. Results showed that the most dominant phyla in *G. Bimaculatus* were Firmicutes and Bacteroidota, while in *L. migratoria* Proteobacteria and Firmicutes. The most dominant genera were Parabacteroides and Pseudomonas in *G. bimaculatus* and *L. migratoria*, respectively. Besides, alpha and beta diversity analysis showed that the diversity and structure of the gut microbiome differed significantly in wild and farmed insects of both species. We isolated 372 strains from wild and farmed insects. In farmed crickets, a higher percentage of microorganisms were able to hydrolyze pectin and starch which can be explained by the inclusion of fruits in its diet, whereas in wild crickets this was cellulose and xylan. From wild *L. migratoria*, a higher percentage of microorganisms were able to hydrolyze the four plant polymers tested than in the farmed ones. Besides, more bacteria hydrolyzed urea in farmed crickets and locusts, while wild locusts and crickets had more microorganisms fixing nitrogen and hydrolyzing uric acid. Finally, the effect of selected strains (Cellulosimicrobium sp, Paenibacillus sp and Pantoea sp) as probiotics was evaluated on biological parameters in both species.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Genetics, age, and environment influence the plasticity of gut bacterial communities in black soldier fly larvae

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Black soldier fly larvae (BSFL; *Hermetia illucens*) are able to feed on and upcycle a variety of organic waste streams. Such plasticity in digesting diverse substrates has often been attributed to the potential presence of a core microbiota in all BSFL. However, there is no clear consensus reported across several published studies, and it is challenging to disentangle the genetic and environmental effects that may influence such phenotypic plasticity. Here, we analyzed the gut microbial communities of two genetically distinct lines of BSFL (wild type vs. lab adapted). Specifically, larvae were reared on ten different diets (based on food waste streams that are common in Southeast Asia). Using high-throughput 16S rRNA gene sequencing, we investigated the influence of diet, genetics, and age of larvae in shaping the gut bacterial communities. We documented that there was significant temporal and environmental plasticity, namely the age of the larvae as well as nutritional properties of diets, that influenced both the diversity and abundance of gut bacterial taxa in the wild type and lab adapted BSFL. However, we did not find core taxa across both genetic lines, and, with a sensitivity analysis, we highlight that previous assignments of core communities can be significantly influenced by threshold limits. We argue that different genetic populations of BSFL differ in their ability to adapt to various diets specifically with respect to plant or meat-based diets.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Feast of the microbes: unravelling bacterial dynamics in black soldier fly larvae rearing

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Black soldier fly larvae (BSFL) rearing has garnered much attention for organic waste conversion and sustainable protein production. During natural decomposition, the microbial communities associated with BSFL have a diverse and important function in the processes of nutrient cycling. In this study, the dynamics of the bacterial community during BSFL rearing days were investigated by amplicon sequencing of partial 16S ribosomal RNA genes, gene expression of carbohydrate-active enzymes (CAZymes), and co-occurrence network analysis. The results showed that microbial communities differed substantially over the rearing days. Out of the 623,305 classified reads in a total of sets of 4 samples with triplicates analyzed, the most dominant phyla identified across the rearing days were *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. The substantial change in BSFL feed microbiome over rearing days is expected due to gradual changes in physical parameters including temperature, moisture, pH, and oxygen availability. Yet, current results revealed that genera of lactic acid bacteria were largely dominating the feed substrate throughout the rearing period (87-70%), withstanding the temperature and pH fluctuations. Gradual change over temperature and other physical parameters favoured an increase in the relative abundance of *Actinobacteria* from day 1 (10%) to day 7 (25%)of rearing. The relative abundance of homo- and hetero-fermentative enzymes were 1.8-2.2 folds higher in the earlier rearing days. Meanwhile, later days of rearing were dominated by nitrate assimilation and chitin degradation-related genes. Understanding this scientific knowledge could be beneficial for the commercial productivity of insect bioconversion. These findings enhance our current understanding of the role of microbiota in the bioconversion of wastes into alternative protein production.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Masters of adaptation: diet-dependent functional variations of the black soldier fly gut microbiome

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Insects play an increasingly important role as alternative source of protein for the sustainable production of food and feed. The black soldier fly (*Hermetia illucens*) larvae (BSFL) are used to replace soy and fishmeal in livestock farming and aquaculture. By using industrial side-streams, low-cost feed sources can be provided for insect farming, thereby contributing to a circular economy. However, many of these side-streams are low in nitrogen and contain difficult to digest fibre e.g. cellulose and pectin. In order to evaluate microbial adaptations to the provided side-stream, we analyzed the gut microbiome (bacteria, archaea and fungi) of BSFL via amplicon sequencing and predicted functions via PICRUSt2. We found pronounced changes in microbial composition depending on diet, e.g. the gut microbiome of larvae fed diets high in protein was dominated by *Enterococcacea* and *Morganellaceae* whereas *Lachnospiraceae* were found in high relative abundance in guts of BSFL fed with high-fibre-diets. Furthermore, potential nitrogen-fixing as well as cellulose- and pectin-degrading bacteria were enriched only in BSFL fed with high-fibre-diets, indicating functional adaptations of the microbiome. Interestingly, however, most of these "beneficial" bacteria were absent in the feed or the eggs, indicating possible other unknown sources of inoculum. Although BSFL can grow on the high-fibre diets, their developmental time was much longer when compared to high-protein diets, indicating slow microbial adaptations to the difficult to digest feed components possibly due to the absence of such beneficial microbes in the feed sources. Our results show that the gut microbiome is one of the most important levers and might be optimized for a more efficient feed turnover to make insect farming more economical.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Virus disease outbreak in *Gryllus bimaculatus* farms in South Korea

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Crickets are valuable as a food source for animals, such as reptiles and fish, due to their high protein content and nutritional value in South Korea. Recently, *Gryllus bimaculatus*, also known as two-spotted crickets, have been showing abnormal symptoms that ultimately lead to the death. Samples were collected from cricket farms at various farms in Korea. Then, metatranscriptome analysis was performed to find the pathogen. Host binding reads were subtracted from the total metatranscriptome data to obtain microorganism reads. Finally, we discovered that the noble densovirus was causing the mass mortality of crickets, and we named this virus as *Gryllus bimaculatus* densovirus (GbDV). Also, the complete genome of the virus was then analyzed and the structural and non-structural protein coding regions was identified. The genome size of GbDV is 5,643 bp, containing five predicted ORFs and the genome includes three NS proteins at the left half of the genome and two structural proteins (VP1 and VP2) at the right hand of the genome. Moreover, we have been tracking the spread of the virus nationwide and its impact on farms. The purpose of this research is to aid in the diagnosis of pathogens that can cause issues in insect farming, ultimately minimizing the damage caused by insect disease outbreaks.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Mutual promotion and restraint between black soldier fly and microorganisms

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The symbiotic or associated microbiota is essential or very important to the development, health, resistance to disease, and environmental adaptation of the host insect. Black soldier fly (BSF) has been considered as promising novel animal protein resources which can convert various organic waste into biomass rich in protein and fat. The interaction between BSF and its microbiota is very worthy of attention and research, and their interactions are very complex and diverse. Microbial signalling that can enhance the mating and oviposition of BSF, and probiotic bacteria that can improve BSF development rate and waste management efficiency have been reported widely. We significantly increased the conversion rate and body weight of BSF by inoculating bacteria isolated from the gut of BSF into the conversion system. In addition, the total nutrient and organic matter content of the substrate decreased significantly and was transferred to the BSF larvae to promote its growth. Moreover, we isolated microorganisms from the intestinal tract of BSF that inhibited Staphylococcus aureus, demonstrating that BSF has an important role in zoonotic pathogens. So far, black soldier fly has showed a very strong vitality, tough but not invincible,it also suffered from disease caused by bacteria. Hence the interaction between BSF and microorganisms has two sides, both promote each other and restrict each other. Here we will systematically discuss the dynamic BSF-microbe relationship from the functional point of view and how the microbiota can lead to alternative valorization strategies for BSF. Emphasizing the importance of revealing the types and roles of microbial interactions for the promotion and development of insect protein industry and applications.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Detection of viruses in black soldier fly using bioinformatic surveillance

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Mass rearing of animals is highly conducive to microbe transmission, which could be detrimental to an industry if pathogens arise. Insects are no exception, and as interest in black soldier fly rearing increases, so should efforts to understand the microbes they encounter, especially in the case of potential pathogens. Viral epidemics in other industrial insects such as crickets suggest that it is important to monitor for emerging pathogens in black soldier fly, which until recently, had no known associated viruses. To understand the viruses black soldier flies harbor, we surveyed 76 black soldier fly gut, frass, and diet transcriptomes from varying nutritional profiles. We detected two known black soldier fly-associated virus sequences and report full genomes of two novel black soldier fly-associated viruses. We phylogenetically analyzed the novel virus sequences and found that they are related to other insect viruses in the families Totiviridae and Rhabdoviridae. Interestingly, the rhabdovirus sequence groups within the sigmaviruses, of which some species are used as models of host-pathogen co-evolution in *Drosophila*. Additionally, we detected a densovirus-like transcript in two larvae samples, which could be of concern to black soldier fly health, but further investigation is necessary to determine the origin of this transcript. Our work shows that bioinformatic surveillance is a useful tool to detect known and novel viruses in black soldier fly and identifies viruses that could have negative impacts on black soldier fly rearing and health.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Isolation of glycine from *Hermetia illucens* L. pre-treated with fermentation using *Lactobacilus plantarum*

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Hermetia illucens is a promising alternative source to produce glycine that play important roles as neutrotransmitter in central nervous system. The protein and glycine content of *H. illucens* can be increased by pretreatment of solid-state fermentation using *Lactobacillus plantarum*. This study aimed to determine the effects of fermentation pretreatment on the crude protein content of black soldier fly larvae, protein recovery, degree of hydrolysis, and glycine content in the protein extract of *H. illucens*. The results showed that the fermentation of *H. illucens* successfully increased the crude protein content up to 3.44%. The crude protein in *H. illucens* was extracted using 0.25 M NaOH followed by hydrolysis using 3% bromelain enzyme to obtain protein recovery and degree of hydrolysis in the range of 35.99-41.60% and 51.52-53.19%, respectively. The protein hydrolysate was then fractionated with a size-exclusion chromatography and the amino acid composition of the fractionated protein hydrolysate was determined using an ultra high-performance liquid chromatography. The results showed that the fermentation was able to increase the glycine content by 1% to 314.95 mg/kg. Hence, pretreatment of black soldier fly with solid-state fermentation using *Lactobacillus plantarum* may be considered as a promising strategy to increase the glycine content of black soldier fly larvae for future application in nutraceuticals.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

The effects of different sources of microbes on the growth and development of the black soldier fly and its applications

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The black soldier fly larvae (BSFL) can transform organic waste into high-end proteins, lipids, and chitin at an industrial scale. However, the effect of exogenous microbes on BSFL growth remains unclear. We constructed germ-free BSFL and sterile materials and used 16 s rRNA and metagenomic sequencing techniques to compare the effects of different sterile and normal materials, as well as egg-carrying microbes on the microbial community of the BSFL and to investigate the role of exogenous microbes in influencing the growth of BSFL. The results showed that exogenous microbes contributed significantly to kitchen materials, the survival rate of germ-free BSFL increased by 26.95%, the length of pre-pupation was shortened by 23.33%, and the average body weight at pre-pupation was increased by 118.50%. Egg-carrying microbes also promoted the growth of BSFL, most significantly in sterile kitchen materials, with a 106.50% increase in mean body mass. Further, 16 s rRNA analysis revealed that specific microbes could assist BSFL from an exogenous environment, among which the Firmicutes is a kind of phylum selected directionally during the feeding process. For the isolation and application validation of intestinal microbes, obtained *Bacillus subtilis*, *Bacillus velezensis*, *Enterococcus ornithischus*, and *Enterococcus wangshanensis* and prepared as additives; germ-free BSFL inoculated with 1% concentration of microbial additives increased survival by 13.00%, pre-pupal duration was reduced by 46.44%, and average body weight was increased by 201.51%. This study revealed the interactions between BSFL and exogenous microbes and provided a reference for further exploring the interactions between BSFL hosts and microbes. Developing an additive of gut microbes for BSFL can help enhance growth and promote a healthy development industry.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Synergistic bioconversion of organic waste by black soldier fly larvae and thermophilic cellulose-degrading bacteria

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This study examines the optimum conversion of Wuzhishan pig manure by black soldier fly larvae (BSFL) at various phases of development, as well as the impact of gut microbiota on conversion efficiency. In terms of conversion efficiency, BSFL outperformed the growing pig stage (GP) group, with significantly higher survival rates (96.75%), fresh weight (0.23 g), and larval conversion rate (19.96%) compared to the other groups. Notably, the GP group showed significant dry matter reductions (43.27%) and improved feed conversion rates (2.17). Nutritional composition varied, with the GP group having a lower organic carbon content. High throughput 16S rRNA sequencing revealed unique profiles, with the GP group exhibiting an excess of Lactobacillus and Clostridium. Promising cellulose-degrading bacteria in pig manure and BSFL intestines, including *Bacillus cereus* and *Bacillus subtilis*, showed superior cellulose degradation capabilities. The synergy of these thermophilic bacteria with BSFL greatly increased conversion efficiency. The BSFL1-10 group demonstrated high growth and conversion efficiency under specific conditions, with remarkable larval moisture content (71.11%), residual moisture content (63.20%), and waste reduction rate (42.28%). This study sheds light on the optimal stages for BSFL conversion of pig manure, gut microbiota dynamics, promising thermophilic cellulose-degrading bacteria, and the significant enhancement of efficiency through synergistic interactions. These findings hold great potential for sustainable waste management and efficient biomass conversion, contributing to environmental preservation and resource recovery.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

The rearing substrate can influence the survival rate and antimicrobial activity in BSF larvae challenged with bacteria

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The larvae of black soldier fly (BSF, *Hermetia illucens* L.) are nowadays gaining increasing attention for their bioconversion capability and the possibility to exploit their biomass for multiple biotechnological applications. The substrates used for larval rearing needs to be carefully evaluated as it can significantly influence the insect growth. The literature reports data on the nutritional needs of the larvae and proper chemical composition of the rearing substrate to obtain optimal growth performance, but more in-depth evaluations on how diet composition affects the immune system of the larvae are needed. This research aimed at evaluating if three diets with different composition (i.e. plant-based by-products (P-BPs): 100% fruit-vegetable; meat-based by-products (M-BPs): 100% meat; mix by-products (Mix-BPs): 50% plant-based by-products and 50% meat-based by-products) influenced the antimicrobial activity of BSF larvae. Last instar larvae were injected with 5 µL of a mix of Gram-positive and Gram-negative bacteria (*Escherichia coli* and *Micrococcus luteus*) at concentrations ranging from 10^4 to 10^8 CFU/mL and larval mortality was monitored every 24 hours for 3 days. Larvae reared on P-BPs diet showed the lowest resistance to bacterial injection compared to those reared on M-BPs and Mix-BPs. Moreover, larvae reared on the three diets and injected with 5 µL of *E. coli* or *M. luteus* at concentration of 10^5 CFU/mL showed a different antimicrobial activity. In particular, the total bacterial load in the hemolymph 6, 14, and 24 h post-infection was lower in larvae grown on M-BPs and Mix-BPs, confirming that these two substrates were able to confer higher resistance against bacterial infection in BSF larvae. The approach herein presented represents a novel, promising method for screening diets that may guarantee appropriate growth performance of the larvae improving, at the same time, their capability to counteract pathogens.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Changes in bacterial community structure across the different life stages of black soldier fly (*Hermetia illucens*)

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The digestive capacity of organic compounds by the black soldier fly (BSF, *Hermetia illucens*, Diptera: Stratiomyidae, Linnaeus, 1758) is known to rely on complex larva-microbiota interactions. Although insect development is known to be a driver of changes of bacterial communities, the fluctuations along BSF life cycle in terms of composition and diversity of bacterial communities are still unknown. In this work, we used a metabarcoding approach to explore the differences in bacterial diversity along all four BSF developmental stages: eggs, larvae, pupae, and adult. We detected not only significant differences in bacterial community composition and species richness along the development of BSF, but also nine prevalent amplicon single variants (ASVs) forming the core microbiota. Out of the 2,010 ASVs identified, 160 were significantly more abundant in one of the life stages. Moreover, using PICRUSt2, we inferred 27 potential metabolic pathways differentially used among the BSF life cycle. This distribution of metabolic pathways was congruent with the bacterial taxonomic distribution among life stages, demonstrating that the functional requirements of each phase of development are drivers of bacterial composition and diversity. This study provides a better understanding of the different metabolic processes occurring during BSF development and their links to changes in bacterial taxa. This information has important implications for improving bio-waste processing in such an economically important insect species.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Crossroads of preference: oviposition and developmental success in black soldier fly rearing

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As the world's population continues to grow, the expected increase in food production, including that of meat, cereals, and fish, will lead to an increase in organic waste. In this context, the use of insects such as the black soldier fly (BSF) has been proven to be a viable solution to address the challenge of degrading and valorizing these wastes, as the larvae can be used as a protein-rich substitute for conventional feed in livestock farming. However, targeted deposition of the largest fertilized egg clutches possible is a key challenge in the industrial breeding of BSF. Our approach involves the identification of chemical signals from microbes that enhance the oviposition behaviour of BSF. This study aimed to achieve this goal by combining egg-laying experiments with semiochemical, microbiological, genetic, and genomic analyses. From a range of organic substrates, we attempted to identify the most attractive one for oviposition by BSF females. We presented the substrates to adult flies in Petri dishes with perforated lids to release odorants and attached egg traps for oviposition. The results clearly show that adult BSF females preferred chicken manure for oviposition. In a follow-up experiment, we aimed to evaluate whether the substrate chosen by adult BSF females for oviposition was also suitable for different developmental stages of BSF. The same substrates as in the oviposition experiments were fed to the larvae to determine their effects on developmental stages. The results showed that the substrate prioritized in the first experiment did not lead to optimal development at any developmental stage – chicken manure slowed down the developmental process from the larval to pupal stage, led to the highest larval mortality, produced smaller adult flies, and resulted in no oviposition and thus no reproduction. Studies such as this are crucial to gain a comprehensive understanding of beneficial insects, such as BSF, and to optimize breeding for wider applications.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Insect probiotic-assisted farming: a promising tool to enhance edible insect health and performances

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Farming of insects intended as feed and food involves different species of interest, among which the yellow mealworm *Tenebrio molitor* and the black soldier fly *Hermetia illucens*, which are the focus of this study. In the last years many works have been performed on the microbial characterization of farmed insects, and several publications have been devoted to the characterization of probiotics able to improve the insect growth, reproduction and health in mass rearings. The aim of this project is to develop microbe-assisted strategies to enhance the growth, reproduction and immunity response of mass reared insects through the administration of probiotic strains. Probiotics administration could be helpful to exploit insect farming, to produce in less time the larval biomass, to sustain rearing and to obtain healthy insects in crowded livestocks. Moreover, the study tends to increase the knowledge on the molecular mechanisms underneath the host-microbe interactions. The study will consider (1) the gut microbiota characterization of insects reared on different diets; (2) the isolation of bacterial strains and their characterization of hydrolytic profile and ability to counteract pathogens; (3) the probiotic administration to insects; (4) the evaluation of insects' performances and health enhancement. The study underlines the importance to promote the development of healthy production of insects as feed and food. The work is funded by 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.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Supplementation of bacterial probiotics to enhance bioconversion ability and growth performance of *Hermetia illucens*

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Microorganisms associated to the insect gut exert important roles for the insect biology, influencing nutrition, immunity, and reproduction. Considering their huge importance, microbial administration as probiotics in insects farming has many beneficial aspects. Among the species currently considered in mass insect farming, the black soldier fly (BSF) *Hermetia illucens* (Diptera, Stratiomyidae) can be efficiently grown on different organic substrates such as those derived from agro-industrial processes. Aim of this work was to evaluate BSF performance on different agro-food by-products, evaluating the substrate impact on the gut microbiome, and to investigate if supplementation with probiotics could improve BSF growth and bioconversion. BSF growth performance was first analysed on okara, brewer's spent grains, potato peels and potato selection waste. Results showed that BSF was able to bioconvert the by-products, although with different degrees of efficiency: while good and rapid development was observed on okara, on brewer's spent grains and potato selection waste, BSF growth was slower. Thus, we decided to apply the bacteria on these two suboptimal diets to observe any probiotic effects on BSF larvae. To achieve this goal, we administered sporeformers and lactic acid bacteria, specifically isolated from BSF gut when reared on a nutritionally complete diet, both considering active and heat-inactivated cells. In few cases the supplementation with active bacteria suggested a possible positive effect, while higher final weight was generally obtained with the administration of heat-inactivated bacteria, but it also took longer for the larvae to reach the prepupal stage. In conclusion, BSF microbial assisted rearing could be an interesting strategy to convert organic wastes into high quality products. The work is funded by 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.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Identification and characteristics of lactic acid bacteria of 12 strains isolated from fermented edible insects

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Edible insects can increase their functionality through fermentation and produce lactic acid bacteria. In this study, we aimed to investigate the suitability of LAB isolated from fermentations of three edible insects (*Tenebrio molitor* larvae, *Protaetia brevitarsis* larvae, and *Gryllus bimaculatus*) as probiotics. A total of 12 strains were identified through 16 s RNA sequencing, and probiotic safety, resistance to artificial gastric acid and bile, and antioxidant activity, including gelatinase and urease tests, were investigated. All strains showed negative activity in gelatinase and urease tests. Regarding the level of gastric acid resistance, the *Pediococcus acidilatici* strain (8.02 log CFU/ml) showed the highest survival rate after 3 hours at pH 3. LAB isolated from mealworms survived at a level of (6.59-7.24 log CFU/ml), LAB isolated from slugs survived at a level of (7.12-8.02 log CFU/ml), and LAB isolated from cricket survived at a level of (7.00-8.08 log CFU/ml). In the resistant bile juice test, 12 strains showed a survival rate of 7.64-9.36 log CFU/ml at bile concentrations above 0.3% (w/v) for 24 hours. The antioxidant activity was measured by DPPH scavenging activity, which ranged from 28.92% to 68.27%, with *Pediococcus acidilatici* having the highest antioxidant activity. These results suggest that strains isolated from fermented edible insects can be considered probiotics with antioxidant activity.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Adaptations of the gut microbiome of black soldier fly larvae (*Hermetia illucens*) to industrial high-fibre side streams

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Due to its high protein content, black soldier fly larvae (BSFL) have a promising role among the insect species farmed as feed for animals. They are considered voracious feeders which consume and break down up to 70% of most organic material. Thus, BSFL is versatile in a range of feeding substrates and their bioconversion rates. In Germany, industrial side streams are available in large quantities and are used inefficiently, which is of interest from the perspective of the circular economy. However, the impact of these side streams' quality and quantity on BSFL is still uncertain. In this experiment, the BSFL was reared on regional side streams, e.g. apple pomace (byproduct from the winery) and potato pulp (starch manufacture byproduct). Both of the side streams are low in fat and protein, but high in fibre. Therefore, it could be a challenge when fed to the animals. Our findings demonstrated that BSFL can be reared on potato pulp and apple pomace, despite the longer growth time (~70 d to reach the prepupal stage) and the final larval weight was ~50% in comparison to the control group reared on chicken feed. In particular, we investigated the adaptation of gut microbiome to the fibre-rich side streams using amplicon sequencing and prediction of microbial functions via PICRUSt2. The results describe the tendency to have higher relative abundances of cellulose- and pectin-degrading bacteria in the guts of BSFL reared on high-fibre side streams than in the control group. This provides the opportunity to further explore the microbiome adaptation and the ability to digest the side streams. Several fungal and bacterial strains with cellulolytic activity were isolated and thus screened for their potential as probiotics.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Ecological role and potential pathogenicity of microbial communities in *Tenebrio molitor* frass from three countries

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Mealworms are increasingly farmed for food and feed, and yield frass (manure), a by-product which has interesting applications as an organic fertilizer. A concern for the presence of potential human and plant pathogens in the manure has however been expressed. To this end, Europe requires thermal sanitation of compost (75 °C, 1 h) but North American countries have no fixed recommendations in their regulations. Nevertheless, manure microbial communities may actually be beneficial for plant growth, improving their nutrition and immune system. Our study aimed at comparing untreated and thermally treated frass from three different countries to see if farming operations or heat treatment could affect the microbial composition of frass. Three frass samples were collected in commercial mealworm farms of Norway, France and Canada. Aliquot of the samples were heated (70 °C, 1 h). All samples underwent shotgun metagenomic sequencing (GenoScreen, France). The ecological role and risk of microorganisms were assigned based on taxonomic identification and raw data were further processed to identify genes of interest (INRS, Canada). Overall, frass bacterial and fungal communities were different between the three different countries. While the heat treatment did not appear to influence the bacterial and fungal composition of frass from Norway, that of French and Canadian frass did vary. Norwegian frass appeared to have fewer potential pathogenic bacteria compared to France, but more potentially antibiotic resistant enterococcus. French and Canadian frass were more heavily associated with nitrogen-fixing mineral solubilizing bacteria. Furthermore, feed and farming conditions seemed to modulate microbial communities as fermented feed was correlated to more acetic and lactic fermentative bacteria. This may bear potential benefits in optimizing upcycling of food waste with edible insects to decrease the carbon footprint of food production.

INSECT BIOTECHNOLOGY, MICROBIOLOGY AND MICROBIOME

Screening the genome of the black soldier fly for enzymatic activity using microfluidics

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The black soldier fly is a promising organism for circular food production and treatment of organic waste. However, knowledge of the fly's genetics, and potential for genetic improvement is lacking. I am using high throughput microfluidic techniques to screen the genome of BSF for enzymes with economically relevant catalytic activity. By using microdroplets in microfluidic devices as in vitro compartments we can analyse the enzymatic activity of 100 million genes per day, making this effort resource efficient (only pL of reagent needed for one assay) as well as fast (.kHz). I am testing for degradation reactions for which assays have already been established in microdroplet format (CAzymes, phosphate and sulfo-transfer, esterases, reductive aminases/IREDs, ketoreductases, peroxidases, plastic degradation: PETases, nylonase), using fluorescence, absorbance and particle scatter assays. Genomic (fly) and metagenomic (fly gut) DNA libraries are inserted into expression plasmids and/or fosmids and tested for the panel of diagnostic degradation reactions. This approach offers and exciting opportunity to directly identify the catalytic activity of enzymes in poorly studied genomes such as the black soldier fly and associated microbiome. This contrasts with traditional approaches to functional characterisation of genomes which rely on sequence comparisons that can only identify enzymes closely related to known sequences in the database.

INSECT HEALTH, BIOSECURITY AND ETHICS

Isolated and identified pathogenic bacteria from black soldier fly larvae with "soft rot" in mass production facilities

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The black soldier fly larvae (BSFL) have the remarkable ability to convert organic waste into high-value proteins, lipids, and chitin on an industrial scale. However, as production capacity is scaled up, health risks to BSFL have also emerged. In this study, we examined the occurrence of "soft rot" disease in mass production facilities, which leads to developmental inhibition and death of BSFL. The responsible pathogen, GX6, was isolated from BSFL affected by "soft rot" disease and identified as *Paenibacillus thiaminolyticus*. Interestingly, higher temperatures exacerbated BSFL mortality and suppressed their growth, while increased substrate moisture has the opposite effect. Additionally, as BSFL age increase, their tolerance to pathogens increases. Upon dissection and examination, it was observed that the middle intestine of infected larvae become swollen and transparent. Furthermore, 16S rRNA gene sequencing analysis of intestinal samples revealed significant alterations in the gut microflora composition of GX6-infected BSFL. Notably, Dysgonomonas, Morganella, Myroides, and Providencia bacteria were more abundant in the intestines of GX6-infected BSFL compared to control groups. This study lays the groundwork for effective control of "soft rot" disease and supports the healthy development of the BSF industry, contributing to organic waste management and the circular economy.

INSECT HEALTH, BIOSECURITY AND ETHICS

Effects of insecticide residues on insects reared for food and feed

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Insects reared for food and feed may be exposed to pesticide residues from agricultural use. Substance-specific legal limits in substrates may differ per jurisdiction, but are generally based on limits established by Codex Alimentarius (CXL). Issues with dietary exposure of reared insects to pesticide residues are twofold. Firstly, this could result in reduced survival and yield. Secondly, transfer or bioaccumulation of substances from substrate to insect biomass could result in non-compliance and/or food or feed safety concerns. The results of 5 experimental studies on the effects of insecticide residues on *Hermetia illucens* (BSFL) and *Alphitobius diaperinus* will be presented. The aim of these studies was to determine the effects of dietary pesticide exposure on the larvae of these species. A variety of insecticides with different modes of action have been assayed, either in isolation or in combinations with up to 4 substances ('cocktails'), in case of BSFL. Concentrations in larval and frass samples were analysed with LC- or GC-MS/MS to assess transfer/bio-accumulation. Statistical differences between treatments and controls were determined. Concentration/response curves were created (PROAST software) for several representative compounds (cypermethrin, deltamethrin, pirimiphos-methyl (PM)). Results showed that concentrations at, or below, legal limits resulted in reduced performance. Effects for deltamethrin were most severe for BSFL, with only 0.04 mg/kg calculated to result in –10% yield – against a 2.0 mg/kg limit in wheat. Combinations of substances ('cocktails') can amplify adverse effects, presenting a concern even in case of lower concentrations. A cocktail of 3 organophosphates (PM, malathion, chlorpyrifos-methyl), each at ~3.3 mg/kg, had the same adverse effect on BSFL as only PM at 9.2 mg/kg, i.e. tripled. No bio-accumulation was observed, but some transfer. These findings have implications in terms of commerce (reduced yields) as well as ethics, since observed adverse physiological effects can be considered detrimental to insect welfare. It is recommended that commercial insect farms monitor pesticide concentrations in feed materials, and that policymakers consider adjustment or establishment of legal limits tailored specifically to reared insects.

INSECT HEALTH, BIOSECURITY AND ETHICS

Practical approaches in contamination management of an open black soldier fly rearing farm

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Managing disease in insect production is a persistent challenge, particularly in the expanding industrial insect sector. Despite the known resilience of black soldier flies (BSF), the transition to larger facilities amplifies biosecurity risks, necessitating robust disease management strategies to ensure the health of the insect population. In a specific incident in Selangor, Malaysia, a BSF open rearing system encountered a significant increase in larval mortality during the 4th to 5th instar stage. Examination of symptoms, including internal body morphology, raised concerns of microbial contamination as the main culprit. To validate this, infectivity tests were conducted, involving the transfer of deceased larvae from affected crates to healthy, uncontaminated ones. The tests confirmed the infectious nature of the disease, with indications of potential vector involvement in the disease transmission. Using molecular methods (16S NGS bacterial sequencing) unveiled a shift in bacterial communities. Opportunistic bacteria commonly found in the gut, including *Providencia*, *Morganella*, and *Dysgomonas*, emerged as possible contributors to larval mortality. Intriguingly, a bacterium from the genus *Leucobacter*, associated with the entomopathogenic nematode *Steinernema thermophilum*, was also discovered in both healthy and diseased larvae, highlighting the intricate dynamics of biological contamination. Further diagnosis with metagenomic shotgun sequencing were done to pinpoint specific causative agents. The overall findings prompted a thorough review of crucial control points, leading to the implementation of specific measures designed to control and eliminate the spread of disease within the facility. This comprehensive approach not only aims to manage the immediate contamination event but also strives to establish a resilient biosecurity framework for sustained and scalable insect farming practices in the industrial sector, where the delicate balance between production efficiency and disease mitigation is crucial for long-term success.

INSECT HEALTH, BIOSECURITY AND ETHICS

BSF health and welfare: emerging challenges for the industry

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Among the growing research conducted on insect rearing (and particularly BSF), one critical challenge is lagging in terms of industrial transfer: the health and welfare of BSF. Indeed, BSF has traditionally been considered highly resistant to infectious diseases and until recently, there were no published reports of naturally occurring outbreaks. As for welfare, insects have been typically classified as non-sentient, thus lacking consciously experienced states such as pain, and given this, falling outside the sphere of moral concern. Recent publications have unravelled these beliefs by documenting outbreaks in BSF rearing factories and a high likelihood of sentience. The aim of this talk is to present these emerging challenges in the context of mass-produced BSF and highlight the remaining scientific gaps. It is based on the review of about 140 scientific publications published until Spring 2023. One on hand, the recent literature exhibits health risks posed by pathogens and exogenous pests. These risks are potentiated by a multitude of factors, and currently, diagnostic and treatment options are immature. Indeed, recently described pathogen and pest outbreaks in BSF caused major losses while novel approaches indicate the existence of BSF-specific viruses. On the other hand, the literature highlights some potential welfare concerns such as thermal discomfort in densely populated bins, while adults still living at the end of a production cycle must be euthanized. Luckily, these two topics are also synergistic; in improving one, gains can be expected in the other. To make progress on health and welfare, we need industry-wide collaborations to prepare guidelines, auditing tools, and research efforts. The goal of this communication is to promote these topics from an industry perspective.

INSECT HEALTH, BIOSECURITY AND ETHICS

Bacillus thuringiensis, an ally in the fight on meal moths in a mealworm farm

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Meal moths such as *Ephestia kuehniella* or *Plodia interpunctella* are common pest species in stores and warehouses but also in mealworm farms. The latter is easy to understand as they flourish in a similar environment and consume similar feedstock as the mealworm larvae. It is presumed that there is no direct effect of the meal moth larvae on the mealworm larvae. Yet, they do compete for food and space, the larvae webbing may disrupt processing and the moths may elicit an allergic reaction. Although decades of research has been done to this common pest species, most of the known ways to eradicate the moths are not usable in a mealworm farm due to the unintended but harmful effects on the mealworms. In this study, the potential of *Bacillus thuringiensis* var. *kurstaki* (Btk) was assessed as these bacteria produce toxins that are specific to the order of Lepidoptera and therefore should not harm mealworms (Order: Coleoptera). Several experiments were conducted to assess the effect of Btk on the meal moth (*Ephestia kuehniella*), mealworm (*Tenebrio molitor*) and any combination there off. This will provide information on the effect of meal moths on mealworms, mealworms on meal moth and Btk on both species. The initial results indicate that Btk (up to 1000 ppm), had minimal effect on the mealworm larvae, especially the final yield per cup. Nevertheless, further research is needed to determine any impact on the smaller life stages or any sublethal effects. As expected, the meal moths were negatively affected by Btk with a variable LC50 between 48 ppm and 332 ppm. Based on these results, the use of Btk in mealworm farms to combat meal moth seems promising. Research is currently conducted to finetune the application and dose of Btk ensuring minimal use while maintaining effectiveness ensuring optimal economic and ecologic use.

INSECT HEALTH, BIOSECURITY AND ETHICS

Scientific Declaration on edible insect sentience and welfare

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In a 2023 Scientific Declaration on Insect Sentience and Welfare, coordinated by Eurogroup for Animals, scientists and philosophers expressed support for increasing research in insect sentience, and for the development and implementation of evidence-based welfare measures in insect farming. This declaration aims at synthesising the most recent neurobiological and behavioural evidence on insect sentience and welfare. While scientific consensus on insect sentience remains uncertain, there is growing evidence suggesting that certain insect orders, including those of some farmed species, may experience pain. Moreover, various insect species exhibit behaviours indicative of cognitive complexity. 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 research on vertebrates has overlooked the vast diversity of invertebrates. 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, gleaned 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 it is crucial for developing updated measures based on the Five Domains of Animal Welfare Model (Nutrition, Environment, Health, Behaviour, and Mental Domains), reinforcing the need for comprehensive and species-tailored welfare practices in insect farming. 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 HEALTH, BIOSECURITY AND ETHICS

Investigating an invisible enemy: the problem of ammonia in black soldier fly larvae

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Insect farming is blooming because these organisms can provide excellent nutrients at low environmental cost. Beyond requiring less water, land, and feed per kilogram of protein produced, insects produce also considerably less greenhouse gases than traditional livestock. Yet, ammonia (NH_3) is a gas that can result from insect farming activities. Type and pH of substrate used to rear the larvae can influence the presence and release of this compound in the air since the dissociation of the ammonium ion (NH_4^+) occurs at high pH. Although ammonia emissions of insect farming can be lower than traditional livestock, they can still be detrimental for the environment, insect breeders, but also insect survival. Larvae can experience different concentrations of these chemicals in industrial settings. A recent study shows that black soldier fly larvae (BSFL) can be exposed up to 83.6 g N/kg larvae. Moreover, substrates pretreated with ammonia decreased BSFL rearing performance by more than half. BSFL can be sensitive to both ammonium present in the substrate and ammonia in gaseous form. Despite these evidences, little is known about the lethal effects of exposure of BSFL to these chemicals. We began to investigate these aspects with particular attention to the effect of 13% concentrated commercial ammonia both in gaseous form (NH_3) or diluted in the substrate (NH_4^+; from 1:1 to 1:6 ammonia:water v:v). Our preliminary results show that BSFL larvae are adversely affected by both forms. All larvae died after three hours of concentrated NH_3 exposure, while 1:1 and 1:2 NH_4^+ concentrations reduced survival (52.94 and 73.53%, respectively) compared to average (92.40%). Given these results, we repeated the trial using a novel and more accurate tool, an accumulation chamber, which is able to estimate ammonia concentrations in detail. We aimed at determining the lethal concentration of larvae for both ammonium and ammonia with greater accuracy. This information is critical to anticipate regulatory aspects, improve our understanding to achieve better welfare in insect production, and design insect mass rearing that limit ammonia emissions.

INSECT HEALTH, BIOSECURITY AND ETHICS

Use of native Algerian herbs to boost the immune response of locusts against bacterial diseases in mass rearing

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In the pursuit of optimizing the mass production of *L. migratoria* and *S. gregaria*, for human and animal consumption, research faced a major obstacle: bacterial contaminations, which led to a significant decrease in survival rates, despite rigorous hygiene protocols. Seeking an alternative to synthetic antibiotics to improve insect health, this study aimed to examine the potential benefits of incorporating aromatic plants from the native distribution regions of these insects in Algeria, *S. rosmarinus* (rosemary) and *A. sieberi* (wormwood) into their diet to enhance their survival against bacterial contamination. Specimens of *S. gregaria* and *L. migratoria* were divided into control and test groups. Control groups received a standard diet (perennial grass with wheat bran). Test group diets were supplemented with dried and ground *S. rosmarinus* or *A. sieberi* at 10% of the wheat bran volume. All groups were exposed to *Bacillus* sp. ingested, previously isolated from the VALCORE laboratory's mass breeding. The pathogen was tested on first-stage larvae and newly emerged adults at a dose of 0.30 mg/mL. Survival was monitored and recorded for both groups over 14 days. Results of the study reveal that introducing *S. rosmarinus* and *A. sieberi* significantly improved survival rates compared to control groups. For *L. migratoria* the survival rate for firsts-stage larvae increased from 30.16% in the control group to 63.83% with rosemary and 65.33% with wormwood, For *S. gregaria* the survival rate increased from 28.75% in the control group to 54.16% with rosemary and 63.75% with wormwood. Similarly, newly emerged adults which were even more sensitive to the bacteria than larvae, also benefited greatly, where survival in the control group for *S. gregaria* was zero and 12.66 for *L. migratoria*, the rosemary-treated groups showed survival rates of 34.16% and 37.75%, while for warmwood 47.5% and 45.33%, respectively. These results suggest that both plants exhibit antibacterial or immune properties at specific doses, assisting locusts in combating infectious diseases such as those caused by Bacillus. A prospective study could focus on identifying the specific active compounds in rosemary and absinthe responsible for this effect.

INSECT HEALTH, BIOSECURITY AND ETHICS

Improving insect welfare in Japan will improve animal and human welfare in Japan

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The Japanese Insect Business R&D Platform and the Ministry of Agriculture, Forestry and Fisheries (MAFF) have developed guidelines for the use of insects as food and feed to ensure the safety of insect production, build trust between producers and consumers, and produce healthy insects. Animal production in Japan has a short history compared to Western countries, dating back to the Meiji period (after 1868), and there are no production guidelines and animal welfare is lagging behind. Insect production, on the other hand, has internationally harmonized guidelines with Europe, and insect welfare is not lagging behind. In Japan, insect production is not an industrial process, but rather a localized, ethical consumption process, which has a positive impact on the environment. Improving the working environment in Japan by including people with disabilities and those who have difficulty participating in society in the labour force will contribute to a stronger guarantee of safety and improved quality in insect production, and will contribute to a review of insect welfare. This interaction will lead to improving animal welfare, which is lagging behind the rest of the world, which in turn will have an impact on the environment. Production safety is inseparable from one-health and product quality is inseparable from one-welfare.

INSECT HEALTH, BIOSECURITY AND ETHICS

Environmental swabbing: a key method for *Acheta domesticus* densovirus (AdDV) surveillance in cricket colonies

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Worldwide, the European house cricket, *Acheta domesticus*, is increasingly being reared for both animal feed and human consumption. Stringent biosecurity measures and good husbandry practices are pivotal to ensure animal health and production safety, security and quality. In this context, pathogens represent a significant threat to industrial farms, which can also lead to considerable economic losses. An outbreak of sudden deaths among crickets occurred within a single box of an experimental rearing facility at the Istituto Zooprofilattico Sperimentale delle Venezie (Italy). An investigation into the potential causes, focused on stress factors common to all boxes, was carried out; however, signs and symptoms of crickets direct the attention to the parvovirus *Acheta domesticus* densovirus (AdDV) involvement. Samples from crickets (eggs, nymphs, adults), from different boxes, and their frass were analyzed, as AdDV transmission occurs through the fecal-oral route. Furthermore, in addition to these usual matrices used to date, environmental swabs were collected from critical points within the rearing room to assess viral particle spread. The presence of AdDV was detected in cricket specimens across all developmental stages as well as in their frass and environmental swabs using real-time PCR (Semberg *et al.*, 2019) targeting two genes of the AdDV genome (the viral capsid protein and a non-structural region), and confirmed by sequencing of amplification products (false positive have been excluded). The implementation of this molecular protocol demonstrated its efficacy in identifying AdDV in several samples, underlying its potential as a diagnostic tool for farm surveillance. Finally, environmental swab testing, a quick and easy-to-use method, proved to be an excellent direct approach for detecting the presence of the virus without sacrificing any specimen and extending the toolbox available for diagnosis and surveillance of insect pathogens. Further investigation into the role of stress factors in AdDV latency and reactivation and the environmental persistence of this virus, particularly within industrial *A. domesticus* rearing, is warranted to mitigate future outbreaks.

INSECT HEALTH, BIOSECURITY AND ETHICS

Insect farming, green protein, and the promise of AI

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Artificial Intelligence and particularly Machine Learning (ML) hold promise for deepening our understanding of insect behaviour and refining our ethics around the treatment of insect livestock. As farmers know, the presence of humans in farms is disruptive and stressful for crickets, mealworms, and adult black soldier flies. Here, I describe our ongoing research using ML models trained by experts to monitor insect colonies and anticipate the activities and needs of the insects in advance, creating an opportunity of more efficient and less invasive care. Based on these data, I describe how ML-enhanced monitoring has the potential to improve labour efficiency and reduce resource use, ultimately reducing the cost of insect rearing at scales from small low-tech farms to industrialised megafarms. Finally, I detail the potential of ML monitoring systems to collect and process behavioural data, deepening our knowledge of the sensory experiences of insect livestock, and ultimately influencing more humane and ethical farming practices.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Exploring the halal status of insect-based foods: challenges, opportunities, and ethical considerations

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In recent years, the global demand for alternative protein sources has led to increased interest in insect-based foods as a sustainable and nutritious option. However, the compatibility of these insect-based foods with halal dietary principles remains a largely unexplored area. This presentation aims to systematically investigate the halal status of insect-based foods, addressing the challenges, opportunities, and ethical considerations associated with their consumption within the Islamic dietary framework. Through a multidisciplinary approach, encompassing entomology, food science, and religious studies, we assess the permissibility of insect-based foods by examining the diverse perspectives within the Islamic community. Since there are different Islamic school of thoughts in Islam, consumption of insect has been a diverse subject amongst the various Islamic schools. Maliki sect is the only school of thought that allows the eating of non-poisonous/hazardous arthropods other than locust which is held permitted by all the schools. After the emergence of halal food sector, the question ascended to the faces of Islamic scholars that either the food ingredients derived out of them would be rendered permissible or not. Even in the era where mankind has started realizing insects as alternative sources of protein beyond the conventional food sources, the issue of their consumption becomes more relevant. This presentation identifies key challenges related to insect sourcing, processing, and the presence of contaminants, while also highlighting potential opportunities for integrating insect-based protein into halal diets. Furthermore, ethical considerations surrounding insect consumption, such as animal welfare, environmental sustainability, and cultural acceptance, are critically analyzed. The findings of this research provide valuable insights for stakeholders in the food industry, regulatory bodies, and religious communities, fostering a nuanced understanding of the Halal status of insect-based foods and paving the way for informed decision-making in the evolving landscape of alternative protein sources.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Towards urban food system circularity: managing food waste in cities with black soldier fly bioconversion

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As urban populations increase, the consumption of food is also rising, along with other finite resources such as energy, water, and land. Scientists, planners, urban designers, and policy makers are advocating increasing circularity in the food system to increase efficiencies, minimise loss of land and improve the nutritional values of food to meet this challenge. One of the biggest hurdles to increased circularity in the food system is food waste. In the light of this challenge, circular biowaste processes, such as black soldier fly larvae bioconversion facilities (BBFs), have gained significant attention for their capacity to tighten the food system loop. Realising the potential of such circular solutions in practical terms, however, involves building an understanding of socio-spatial-operational challenges and synergies arising from introduction of 'novel' solutions into the urban environments. This paper presents an integrated framework for circular bioeconomy of decentralised BBFs for dense cities such as Singapore. The framework is drawn from on-site investigations of BBFs coupled with existing theoretical and empirical findings. The paper complements the existing approaches by looking at aspects of land use complementarity (spatial), community engagement (social), and operational policy and regulations of BBFs and their inter-relationships. Key findings of the paper include (a) untapped potential for BBF integration with urban initiatives like community gardens, local food outlets, and schools to enhance neighbourhood food recycling; (b) significance of community engagement and awareness campaigns; and (c) presence of a regulatory vacuum concerning insect-based waste processing in cities, particularly regarding operational parameters around drainage, pest control, and land-use zoning. Using the framework presented in this paper we discuss the linkages among the key spatial, social and operational factors, and identify the important knowledge gaps and focus areas for future research to facilitate innovative and circular urban food waste solutions.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Challenges in developing Japanese insect production guidelines and their implementation in society

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The Insect Business Research and Development Platform (IBRP) in Japan has formally invited IPIFF speakers to engage in active discussions regarding developing and implementing insect production guidelines in Japan. During the talks, the speaker highlighted the significance of transparency, legislation, government cooperation, and the country's international position in successfully executing the project. Japan has an eco-food guideline developed by the Ministry of Agriculture, Forestry and Fisheries (MAFF), which is gaining international recognition. The guideline will also be functional and pragmatic for controlling the safety of insect feed and food regulation. Recently, the IBPF has successfully published guidelines and has a central role in preparing, releasing and contributing insect production guidelines to ensure the safety of crickets, BSF, silkworms, mealworms, and houseflies for food and feed ingredients. Unfortunately, in Japan, the absence of a ministry to manage the insect production area means the private sector continues to manage safety on its initiative. With companies complying with the insect production guidelines, initiatives contributing to a new resource cycle have been initiated and carefully implemented in Japanese communities and societies under administrative control in Japan. This study will present an example of Japanese research carried out in collaboration with prefecture government veterinarians on the safety of insect feeds and edible insects, which are increasingly being used internationally, and will also discuss the prospects for the future.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Insects as food and feed in Quebec: a new strategic planification for 2023-2026

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In Quebec (Canada), according to an updated portrait of the edible insect industry, 30 insect producers and 16 processors were in activity in 2022. Although the edible insect industry is rapidly expanding, its situation is still precarious. In recent months, some companies have made important announcements or have greatly succeeded in up-scaling their production. However, many companies are still at the stage of demonstrating their business model, and others have ceased their activities. Several challenges such as regulations, process optimization, supply, social acceptability, training of qualified workforce and access to markets must be addressed. The Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) promotes the establishment of innovative industries by funding and overseeing the formation of a concertation table which allows industry collaboration and optimized industry development. The concertation table, unique in Canada, regroups 41 stakeholders all along the industrial chain (waste management, insect production and processing, distribution, research, technology transfer, education, outreach organizations and government). In 2023, the TFIC has identified and implemented a new strategic planification for 2023-2026, structured on three main axes (coordination, business development and market development) and including 42 objectives aiming to increase production volumes; implement automation and integration of new technology in insect farms, develop standardized methods and ensure the diffusion of a guidance document on the best practices in quality and hygienic insect production. Several working committees (sector development, quality assurance and transformation, communication and public relations and frass) have been put in place to tackle common obstacles, promote networking and produce open-access documentation. Noteworthy accomplishments include a document on frass definition and application in Quebec, the hosting of transfer events, and to production of a web page on frequently asked questions. The activities of the TFIC should contribute to the sustainable growth and perennity of insects as food and feed in Quebec.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Assessing and managing the risks of *Bacillus cereus* in frass

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Insect manure, called frass, is a valuable fertilizing product that can be used in organic agriculture and gardening. It is also a voluminous by-product of edible insect production which needs to be upcycled by farmers. Its rich microbial diversity may be beneficial for soil and plant health. However, to mitigate the risk of potential pathogens, Europe requires thermal sanitation which is costly, labour and energy-intensive. The European SUSINCHAIN project recently conducted a clinical and microbiological safety evaluation for frass. Amongst all the risks reviewed, they suggested that *Bacillus cereus* is probably the greatest biohazard of insect frass. *B. cereus* is a complex group of bacteria comprising closely related taxa (*B. cereus*, *B. anthracis*, *B. thuringiensis*, *B. toyonensis*, etc.). Amongst these, some can be opportunistic pathogens of humans, associated with emetic and diarrheal diseases. These may in rare cases lead to death, especially in vulnerable populations. On the other hand, some taxa have useful applications in plant and animal health. *B. cereus stricto* sensu is very common in food, soil and different types of fertilizers present on the market, including heat pelletized chicken manure. However, there is no requirement to test for the presence of *B. cereus* in fertilizer in Canada, United Stated, Europe and Australia. Correctly identifying *B. cereus* group species – or the presence of genes coding for toxins – requires genomic tools which are rarely available in commercial quality control laboratories outside of academic institutions. Until easy to use and affordable diagnostic tools are available, a first step in risk management could be the inclusion of recommended use and warnings on frass labels to educate end-users of fertilizers. The development of appropriate quality control criteria for the safe use of frass is essential for the adoption of regulation which will guide the safe valorization of this novel fertilizer, without risking to lose its beneficial microbial community or make insect protein less competitive in the animal protein sector.

INSECT QUALITY CONTROL, LEGISLATION AND POLICY RECOMMENDATIONS

Prediction of insect fat content in salty doughs using handheld infrared spectrometer and partial least squares regression

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Insect fat content can vary depending on the insect species, the development stage, their diet, the way they are processed (thermal, mechanical), and the protocol used for fat extraction. Accurate and rapid determination of fat content is of great economic importance to stakeholders in the insect supply chain. Current assays rely on solvent extraction-gravimetric method to detect the level of crude fat in products and GC-FID or GC-MS for their fatty acid profiling. These methods are time-consuming, expensive, and labour-intensive, requiring complex procedures of sample pretreatment and well-trained technicians to operate the instrumentation. The present study describes a new approach to predict the amount of edible insect lipids (*Tenebrio molitor* and *Alphitobius diaperinus*) and the composition of the lipids added in salty dough using handheld near infrared (NIR) spectrometer combined with multivariate analysis. Several doughs were made using chickpea flour (46.3%wt) water (39.3%wt), curry, salt and different quantities of olive or sunflower oil (up to 11.6%) and insect lipids (up to 11.6%). The crude fat level was obtained by Soxhlet and fatty acid composition analyzed as fatty acid methyl esters was attained by GC-MS. Spectral data was acquired using a handheld FT-NIR scanner (1,350 to 2,550 nm) using 2.5 g of dough placed in a petri dish. Collected spectra were used to develop partial least squares regression (PLSR) model to quantify the amount and type of edible insect fat added to the dough. PLSR models exhibited excellent signal-to-noise ratio and good linearity, predicting insect fat content and type of fatty acid present in doughs with strong correlation ($R_{cv} \geq 0.85$) and low standard error of cross-validation. This research has shown the potential of using handheld NIR devices combined with multivariate analysis to predict the amount of insect fat added in complex matrices.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Towards low-carbon insect-based bioconversion of organic waste

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The resource utilization of organic waste streams is an important part of the global circular economy. Black soldier fly (BSF) bioconversion, as an effective method for high-value utilization of organic waste streams, has great carbon emission reduction potentials. The CH_4 and N_2O emissions from treating one ton of food waste using BSF bioconversion are equivalent to 0.38 kg CO_2. Currently, it is difficult to evaluate the carbon footprint of BSF bioconversion industry due to the lack of large-scale data samples. Therefore, there is an urgent need for an industrial pilot to gradually establish a dedicated carbon footprint assessment method through data collection and quantification of the BSF bioconversion process. This work first proposes a method for measuring the direct carbon emissions during the BSF bioconversion process in a closed experimental system, where a functional microbial agent, *Ochrobactrum* sp., is added to help further reduce the process' carbon emissions. Then, the proposed approach is applied in an industrial pilot, which is capable of processing 50 tons of food waste per day. The application shows that the total direct carbon emission of the sole BSF bioconversion process is 62.75 kg per ton of food waste, which is 65.71-80.52% less than composting. This reveals its great potential for carbon emission reduction. Moreover, after adding the functional microbial agent, the process' carbon emission can be reduced by an extra 6%. With the industrial pilot, by enrichment culture, selective culture, purebred isolation, propagation, and systematic testing, it is possible to screen other functional microbial agents that can further help the BSF bioconversion process reduce carbon emissions. In conclusions, the measurement results reported in this work demonstrate the high carbon emission reduction potential of industrialized BSF bioconversion. This work provides a basis for further research on standardizing the carbon footprint calculation of large-scale BSF bioconversion factories.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Social LCA of a small-scale insect-fed broiler farm

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While environmental life cycle assessment (LCA) and life cycle costing (LCC) are mostly standardized, standardization of social LCA (SLCA) has proven difficult. The methodology applied differs from case to case and thus impedes comparisons and conclusions. This research aims to contribute to the standardization of SLCA in small-scale farming and showcases the use of the developed SLCA methodology in small-scale black soldier fly larvae (BSFL) farm, and small-scale broiler farm which supplements the broiler feed with live BSFL. For both the insect farm and broiler farm 2 scenarios were considered (with predominantly manual production, and predominantly automated) with 2 additional scenarios for the broiler farm (with and without BSFL supplementation). The study followed the cradle-to-farm gate, both for BSFL and for broilers. The grading of social risks was done by assigning a grade between 1 (committed) and 5 (not assessed – unreliable sources) for each of the previously defined 8 categories. The methodology was mostly developed by the combination and adjustment of the literature data, while the farm data from the farms was collected from the experimental farms of the project partners. Following this methodology, it was observed that the introduction of insects did not have a significant impact on SLCA, increasing the results by 0.12 and 0.13 points for manual and automated broiler rearing, respectively. Conversely, a clear positive impact of automatization was observed, leading to a difference of 1.07 points in the case of BSFL rearing, 0.72 points in the case of BSFL-supplemented broilers, and 0.73 in the case of control broilers. The decisive categories were Health & Safety (which included the risk of allergenicity of handling insects), Working hours and Equal opportunities/Discrimination. It can be concluded that, while BSFL inclusion did not lead to a decisive change, an improvement of the social sustainability of insect and broiler farming can be achieved through an increase in the level of automation.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Putting a number on insect welfare! How technology can help. Presenting results from Insect School case study, Part 1.

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Animal welfare is not yet the biggest topic of concern in our industry. Profitability is. Nevertheless, everybody understands the topic of welfare will become bigger and insect production will become more sensitive to this external influence. As the industry reaches maturity, we want to avoid that it is exposed to challenging comments on animal welfare. Focus on money first and welfare second may to many make short-term sense, but could lead us down a road we should try to avoid. So let us take one step back and look further into animal welfare. Covered under the banners of physiological well-being, behavioural expression and environmental conditions. These three aspects are daily attention points for a farmer, consciously or not. An animal that experiences good welfare performs better, is more profitable and has lower environmental impact. A successful farmer knows this so always has the highest care for their animals, the reason why he/she became a farmer in the first place. The problem we face is that animal welfare like sustainability is always a discussion point based on supposition and emotions. This unfortunately is the tangle the current livestock industry has got itself into with NGOs and ultimately society who make claims not based on facts. We in the Insect industry can learn from these mistakes and take a different route. Let's try to objectify it. To use technology and daily observations that can be used to score welfare. Information that can not only be used to improve production processes in the name of animal welfare, but to also be used for sensible dialogue with other industry stakeholders. To put our motivation and dedication into numbers. We combine current scientific thinking with accepted protocols such as e.g. Bramells five freedoms and factor in known parameters based on genetic needs. We then build a data model to register our findings against these, daily. The definition of animal welfare itself is clearly expressed in a presentation by Dr. Meghan Barrett from Indiana University Indianapolis. Part 1 focuses on how we can put animal welfare into practice. Using the 5 freedoms and leaving us with a measure of welfare. Part 2, being a separate abstract, will present results of a case study.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Incorporating insect welfare into IAFF life cycle assessments

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IAFF life cycle assessments (LCAs) attempt to account for the full environmental cost of their products. LCAs can be important parts of policy-making, informing the decisions of those who have to decide which product categories to encourage discourage. The UK's Department for Environment, Food & Rural Affairs (DEFRA), for instance, has expressed interest in having a LCA for BSFL production as it considers whether and, if so, how to regulate the developing IAFF industry. However, while insects are generally viewed as components of the environment, insect welfare is rarely included in LCAs, despite the recent development of methods to incorporate it: see, e.g. Scherer *et al.* (2018), Tallentire *et al.* (2019), and Budolfson *et al.* (2023). These methods allow for the possibility of principled trade-offs between welfare impacts and other environmental impacts, such as water usage and GHG emissions. Thus far, however, they have not been extended to insects or applied in the context of IAFF. The purpose of this presentation is twofold: first, to extend existing approaches so that insect welfare can be incorporated into LCAs; second, to develop some models that illustrate the potential impacts of incorporating welfare into IAFF LCAs. References: Budolfson, M., Fischer, B. and Scovronick, N. 2023. Animal welfare: methods to improve policy and practice. Science 381: 32-34. https://doi.org/10.1126/science.adi0121; Scherer, L., Tomasik, B., Rueda, O., *et al.* 2018. Framework for integrating animal welfare into life cycle sustainability assessment. Int J Life Cycle Assess 23: 1476-1490. https://doi.org/10.1007/s11367-017-1420-x; Tallentire, C.W., Edwards, S.A., Van Limbergen, T., *et al.* 2019. The challenge of incorporating animal welfare in a social life cycle assessment model of European chicken production. Int J Life Cycle Assess 24: 1093-1104. https://doi.org/10.1007/s11367-018-1565-2

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Willingness to pay for insect-based feeds in eastern Africa

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Large-scale production of insects for feed is garnering international attention as an effective approach to repurposing organic waste into high-quality feed proteins. Governments are providing strategic policy support by establishing standards. Despite these initiatives, empirical evidence on farmers' intention and willingness to pay for insects for feed remains limited. This study assessed farmers' intention and willingness to pay (WTP) for insect-based feed in eastern Africa. WTP was elicited using an open-ended contingent valuation method from 914 poultry farmers in Ethiopia and Rwanda. We find that farmers have a good understanding of insect-based feed, and some (4-7%) have tried using it. About 98% of them are willing to use insect-based feeds and they are willing to pay about US $0.15 per kg in 2022 purchasing power parity price (US $0.14 per kg in Ethiopia and US $0.19 per kg in Rwanda). The WTP offered by farmers in Rwanda is comparable to conventional feed prices at the time of the survey. However, the interviewed farmers in Ethiopia offered prices lower than existing conventional feed. The low valuation of insect-based feeds in Ethiopia could indicate the need for strategies to enhance farmers' knowledge of insect-based feeds. Regression analysis further reveals that farmers who follow good poultry management practices (e.g. vaccination and disinfecting the farm) provided a higher WTP. This may indicate that knowledge diffusion on insect-based feeds may need to focus on these farmers as an entry point.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Utilisation of agri-food side streams in insect-feed-animal chains as a sustainable circularity strategy

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Insect production for food and feed is facing numerous challenges associated with potentially high costs, environmental impact and a lack of optimization in production. Particularly, these limitations are associated with the legal limitation in European Union on the feeds allowed for insects as farmed animals. Considering the challenges, an assessment approach of suitable agri-food side streams is needed. The proposed assessment matrix approach is aimed to account for the multiple criteria (legal, nutritional, safety, availability and seasonality, cost, and environmental impact) as a basis for the preselection of feeds suitable for different insect species. Moreover, the matrix-based assessment approach allows for further integration in multicriteria analysis (e.g. multi objective optimization) and larger scale economic scenario analysis. The study, performed in the scope of PRIMA funded ADVAGROMED and CIPROMED projects, presents examples of the matrix approach application for the streamlined determination of suitable side-stream feeds. Further scenario analysis included the economic and environmental effect consideration on economy level. It included the potential of use of some organic side streams in insect feeding to be associated with the mitigation of the impact and even resulted in environmentally positive scenarios. As a case study, it was determined that utilization of agri-food side streams from a hypothetical Mediterranean region (city) with 50,000 people can support the daily production of 3.6-7.1 ton dried *Hermetia illucens* biomass with the daily operational costs of €4,500-5,280. The two potential effects accounted for were waste reduction and return of nutrients to food systems in the form of fertilizers, feed and food. This study account for waste reduction by insects and the potential of insects for nutrient circularity.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Evaluating contributions of insect species to sustainable agri-food systems: a multidimensional approach

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Insects provide multiple ecosystem services – as well as nutritional benefits – which may help meet future food demands in a more sustainable manner. We evaluate 106 insect species for their potential contributions to sustainable agri-food systems using the Integrated Insect Evaluation Framework, qualitatively assessing their productivity, ecosystem services and potential contributions to sustainability. Varying performance among species clusters highlights the need for strategies for using them in agrifood systems tailored to each species. Blattodea, Diptera, and Coleoptera, owing to the extensive research and widespread utilization of many species within these taxa today, exhibit high productive potential for use as food, feed, biocontrol, and/or bioconvertors. Hymenoptera, Coleoptera, and Orthoptera show high score for ecosystem services and sustainability, with some species primarily playing key roles in pollination or biodegradation. Social and economic challenges as well as knowledge gaps are also identified. Overlooked wild insects, including some Apidae, Stratiomyidae, and Tenebrionidae species, may contribute to pollination, bioconversion, and other services, such as food/feed production, indicating the need for bioprospecting. This paper explores the potential of different insect species in terms of productivity, ecosystem services, and sustainability, advocating for a holistic understanding of their potential through collaborative evaluation addressing knowledge gaps and quantifying social and economic aspects of ecosystem services. Multidisciplinary research is crucial to comprehending the role of insects in sustainable agri-food systems.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Enabling circular economy in the Italian agrifood sector: determinants of farmers on insect biorefinery acceptability

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In a world with finite resources, repurposing food loss and waste (FLW) is crucial. Insect biorefinery (IB), an emerging circular bioeconomy approach, plays a pivotal role in upcycling nutrients from FLW. The European insect industry's sustained leadership and integration with the bioeconomy depend on implementing symbiotic production models through site selection and partnerships. Presently, only 8 studies (4 European, 3 African and 1 South America) have explored the acceptability of stakeholders. This Italian pilot study is the first to focus on potential Upstream Stakeholders (US) – defined as the one involved in the earlier stages of the supply chain, such as suppliers – addressing a critical literature gap. This research explores the factors influencing the willingness of potential US to adopt IB. Preliminary analyses probed the willingness of companies to participate in the survey, determining a representative at the level of production heterogeneity sample of 31 SMEs who were administered a survey with 23 questions divided into 4 sections. R software was used to carry out a Principal Component Analysis. It considered five components: (1) company dimension, (2) attitudes towards by-products management on-farm, (3) attitudes towards by-products management off-farm, (4) predisposition toward innovative management methods, and (5) interest in insect rearing. Components 1 and 2 explained 79% of data variances, rising to 93% with n. 3. Component 1 was inversely correlated with 4 and 5. This is potentially influenced by prevalent funding opportunities in the Italian and EU contexts, encouraging innovative practices in small and medium-sized enterprises. Identified barriers to adoption include misinformation, anticipated negative media impact on branding, and resistance to changing by-product management practices. This pilot study serves as a foundational exploration, offering insights into IB acceptability among potential US. It offers valuable insights for future in-depth studies, shaping the discourse on the acceptability and integration of IB within bioeconomy.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Developing an environmental assessment framework for an insect farm operating in circular economy: a case study

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Circular food economy, particularly through insect farming using food waste, has gained increasing attention in the past few years because it could potentially reduce the environmental impacts of food production by repurposing food waste as insect feed while providing an alternative source of protein. Although the environmental advantages of insect farming are well-established, limited research exists on insect farming in colder regions with cleaner grid electricity, such as Quebec, Canada. In this paper, a framework is developed to evaluate the greenhouse gas emissions of a mealworm farm in circular economy. The benefits of circular economy are assessed through multiple different feed acquisition scenarios (virgin feed and feed diverted from composting, anaerobic digestion, and landfilling), and the benefits of insect farming are assessed through a comparison with other sources of protein. The framework is composed of five stages: feed acquisition, transformation, heating, transportation, and fertilizer production. Results show that farming insects using virgin feed emits +14.94 kg CO_2eq/kg protein while using diverted food waste as feed eliminates the need for conventional waste management and leads to a reduction of greenhouse gas emissions of -1.54, -2.48, and -16.39 kg CO_2eq/kg protein for food waste diverted from composting, anaerobic digestion, and landfilling, respectively. The findings also reveal that mealworm farming with virgin feed generates greenhouse gas emissions which are lower than beef and pork but comparable to chicken production. This study demonstrates the environmental superiority of insect farming, with or without food waste, in Canada.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Mass-based and nutritional Life Cycle Assessment (nLCA) of crickets as human food

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Crickets offer a sustainable protein source for human consumption and animal feed, especially with the European Food Safety Authority's approval of house cricket (*Acheta domesticus*) for human consumption. The imperative to enhance acceptance in Western countries is underscored by scientifically supported arguments. This study uses a Life Cycle Assessment (LCA) to analyse the environmental implications of large-scale edible cricket powder production in Vietnam. Data on the essential amino acid profiles of defatted cricket powder were collected and evaluated based on the Digestible Indispensable Amino Acid Score (DIAAS) and digestible protein content. Three functional units were considered: 1 kg of cricket powder, 1.26 kg of cricket powder with 1 kg of protein, and 1.39 kg of cricket powder with 1 kg of digestible protein, enabling comprehensive environmental impact comparisons. LCA results for the three functional units were compared with those of a conventional protein source, using the Environmental Footprint 3.1 methodology and various impact assessment methodologies. Findings revealed that incorporating cassava top (leaf) in cricket feed reduced environmental impacts by -0.453 kg CO_2 eq, a significant 18.54% decrease, with 80.90% of feed impacts attributed to soybean meal. Overall carbon emissions for 1 kg of fresh cricket were 1.99 kg CO_2 eq, mitigated to -0.185 kg CO_2 eq (8.50% reduction) by utilizing frass as a biofertilizer in nearby farms. Environmental impacts associated with 1 kg of cricket powder were 12.1 kg CO_2 eq by edible mass. When assessed by 1 kg of protein, the result was 11.8 kg CO_2 eq, and with 1 kg of digestible protein, it was 11.4 kg CO_2 eq, with 65.98% of total impacts attributed to cricket feed. Comparative analysis with "whey protein concentrate" (WPC80) showed a significant 73.67% reduction in environmental impacts for cricket powder, providing insights into its environmental performance and emphasizing potential sustainability advantages.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Circular integration of insect bio-converting food waste into protein: a Life Cycle Assessment on black soldier fly

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In recent years, black soldier fly (BSF) has gained significant research attention for its potential in waste management and high-protein feed production. Experimental trials across insect species consistently identify BSF as the most efficient biowaste-converting species. A study estimated that with 10 tonnes of food waste (FW) input, 300 kg of dried larvae and 3,346 kg of composed are produced. The study, performed in the framework of ADVAGROMED and CIPROMED projects, aimed to define the potential for the food waste circularity of BSF through Life Cycle Assessment (LCA) modelling based on published and experimental data. Previous LCAs highlighted energy consumption and global warming potential (GWP) as primary concerns. The LCA conducted in this study assesses the environmental impact of BSF bioconversion of various FW. A functional unit of 1 ton of FW bio-digested was used as input; 1 kg of protein for food and feed and 1 kg of frass for soil fertilizer were also considered. Processing 1 ton of food waste resulted in a cumulative impact of 27.5 kg CO_2 for GWP, 205 MJ in Energy Use, and 0.703 m2a in terms of Land use. Electricity constitutes the main influencing parameter for GWP contributing over 70% of emissions. Substituting conventional fertilizers with insect frass also shows a reduction in fossil depletion by 3%. Additionally, when using feed substrates such as abattoir waste or mixed waste fractions it enhances conversion efficiency and reduces its environmental impact; prolonged processes and development times associated with single substrates result in elevated costs and higher GWP. Feeds collected from vegetable overproduction auctions show high waste reduction and survival rates on insect larvae, but protein content was lower. This emphasizes the importance of evaluating substrate parameters in bioconversion technology; recirculating food waste nutrients using insect technologies depends on factors such as initial nutrient richness, insect conversion efficiency, environmental impact, and usability of derived products.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

What's the value of insect protein?

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What's the value of insect protein and perhaps more broadly insect-based ingredients? What do customers get when they purchase insect protein? Why is there seemingly such a variation in market prices and perceived value? In this study, we investigate the value of insect meal derived from black soldier fly larvae based on economic and commercial, nutritional and functional and sustainability perspectives and contrast some of the considerations *vis-a-vis* premium fishmeal, an often referenced benchmark product. For the economic assessment, the prices, applications, volumes, quality and market drivers are evaluated under special consideration of actuals of the past five year period. For a nutritional and functional assessment, we focus on a comparative value model approach and reference products and applications beyond fish meal. Finally, for sustainability, we review existing LCA and impact assessment results and methods and the value mechanics they drive. We finally attempt to offer an integrating perspective of the three dimensions to the question 'What's the value of insect protein?'. The results indicate that insect protein meal has a distinctive nutritional profile in nutrients, but more importantly in functional claims supported by an increasing body of published literature; however when insect protein is sold as a macro-nutrient, a pricing benchmark with other animal proteins should be maintained for competitive value.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Entwined evolution: how innovation networks foster collaborative growth in east Africa's edible insect value chains

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Insects are a viable alternative animal protein source contributing to food and nutrition security. However, little is understood on the edible insect innovation networks, including the actors, their roles and linkages and how this supports or constrains co-evolution and value chain development. This study aimed at evaluating the innovation processes and networks of the value chain in Kenya and Uganda. Data was collected in Masaka, Uganda, and western Kenya from 220 cricket farmers, 440 non-cricket farmers. Secondary and primary data from key innovators were included. The research, utilized a descriptive research design and employed Social Network Analysis and Exponential Random Graph Models to characterize innovation processes and analyze networks. As per the research most edible insect innovators are linked to the supply side. Despite a perceived market readiness, there exists a potential gap between awareness of market opportunities and actual sales realization. Respondents, however, exhibited a strong consensus, expressing a positive inclination and readiness to engage in farming activities. The institutional networks exhibited a density of 0.5588235 with complete reciprocity. In the farmers' network, total degree centralization values were 0.4784946, 0.2444444, and 0.6045229 for Kisumu, Siaya, and Masaka, respectively. The affiliation network's eigenvector centrality values were 0.1282051 and 0.1120563 for Kenya and Uganda, underscoring the significance of specific nodes within these networks. While there is a high level of mutual cooperation within the institutional network, weak linkages are observed in the affiliation network. Recent collaborations between research organizations and the private sector show potential to boost demand, accelerate innovation, and improve markets. The paper emphasizes the importance of recognizing and promoting co-evolution and interactions among actors.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Making better bugs: improving how we raise black soldier flies for a sustainable tomorrow

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The black soldier fly, recognized for its sustainability, undergoes testing in production settings as the industry evolves. Our research prioritizes eco-friendly alternatives to minimize environmental impact. Through renewable resource integration, optimal larval nutrition, and insect genetic advancements, we drive a transformative shift in insect cultivation. This study offers valuable insights, highlighting the black soldier fly's pivotal role in sustainable agriculture. Our analysis of large-scale *Hermetia* larvae production involves two main steps: examining production data and modelling it in SimaPro 9.5.0.2 software for impact analyses. Using Life Cycle Assessment (LCA), we evaluate environmental impacts, focusing on climate change, water scarcity, and land occupation. The methodology used for impact assessment was IMPACT 2002+. The system boundary covers raw material extraction, processing, transportation, and disposal, with the primary functional unit as 1 kg of fresh matter insect. Findings show significant reductions in environmental impacts compared to previous production systems. For instance, carbon emissions (kg CO_2 eq) reduced from 0.020-0.01123 for insect frass-based fertilizers, 0.402-0.198 for fresh insect production, 0.439-0.217 for fresh *Hermetia* puree, 0.907-0.477 for *Hermetia* fat production, and 1.149-0.832 for *Hermetia* meal production. Land use (m^2 org.arable) reductions range from 0.020-0.00004 for insect frass-based fertilizers, 0.461-0.0007 for fresh insect production, 0.445-0.001 for fresh *Hermetia* puree, 0.898-0.0102 for *Hermetia* fat production and 1.137-0.0178 for *Hermetia* meal production. Utilizing feed savings (diet optimization) results in a 64.54% reduction in total carbon emissions. With a total reduction of 70.74% in non-renewable energy use, carbon emissions of different products reduced to 63.59% on average. Results are from Protix, a company in Bergen op Zoom, the Netherlands.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Life Cycle Assessment and environmental performance of insect-based industries: a focus on *Tenebrio molitor* and potentia

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The use of edible insects as a food source for animals and humans has emerged as an issue of worldwide interest, due to global population growth, increasing demand for and rising cost of animal protein, food insecurity in some regions of the world and increasing environmental pressures. However, this industry is faced with the triple challenge of meeting environmental performance requirements in its production, ensuring product quality, and maintaining economic competitiveness in a changing context. While technical solutions have been identified to address these challenges, the question of their sustainable implementation still needs further exploration. A crucial research question concerns the environmental performance of the industry and its impacts compared to other plant or animal-based sectors. While some literature addresses this topic, published results spark debates and inquiries. This study focuses on Life Cycle Inventories (LCIs), which play a decisive role in Life Cycle Assessment (LCA) since the resulting impacts are directly linked to LCIs. Our work examines the LCA of the *Tenebrio molitor* species, considering a scope encompassing both breeding and the transformation of larvae into protein flours. To better guide the inventories required for this LCA, we conducted a Monte-Carlo sensitivity analysis to specify the relative importance of different inventories. This approach applied to the *Tenebrio molitor* larvae could be extended to other insect species. The scientific significance of this study also lies in the potential improvement of future LCAs, aiming to ensure a more accurate representation of the insect industry as a whole.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Biological invasion risks of insect farming

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Insects are renowned for their successful invasions across diverse ecosystems, resulting in significant ecological and economic damages. Consequently, the industrial rearing of insects for food production poses a new dimension of global change: the risk of exacerbating biological invasions. Indeed, by farming non-native species, insect farming could inadvertently promote biological invasions if individuals escape from farms. Our study aims to anticipate both current and future potential regions for invasion by already farmed insect species, as well as areas suitable for farming edible species. To achieve this, we utilized species distribution models incorporating occurrences of 16 farmed species (including *Acheta domesticus* and *Tenebrio molitor*) and 89 edible species, alongside environmental variables that characterize species distribution (e.g. temperature, wind, humidity, net primary production). Through a combination of models including MaxNet, Random Forest and XGBoost, we generated comprehensive maps highlighting suitability areas for each species. Our findings reveal significant hotspots of suitable areas for farmed species, mostly in Europe, North American and East-Asia, with variations among species. This indicates the need for caution in breeding these species in such regions and to select the farmed species accordingly. Moreover, we identify several farms breeding insects in high-suitability areas for these species, highlighting a heightened risk of biological invasion of these species. By evaluating these maps, we contribute to the understanding of sustainable insect farming practices and provide guidance on where species, whether already farmed or new, could be farmed without risk of causing biological invasions.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Revolutionizing BSF farming: how breeding and rearing decoupling unlocks new horizons in insect production

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In contemporary industry, specialization and focus are key drivers of success, and this concept is evident in the black soldier fly (BSF) farming sector. Historically, BSF farming has been predominantly vertically integrated, with a majority of black soldier fly larvae (BSFL) produced by a single entity encompassing all stages from breeding to processing. This integrated approach stemmed from the lack of reliable external sources for BSF breeding outputs, such as eggs or neonates. However, recent advancements in technology have facilitated the development of methods for the effective reproduction, hatching, counting and transportation of BSF eggs and neonates. This innovation has significantly enhanced survival rates during transit to remote farms for subsequent growth and processing. In addition, it enables us to stock ready-to-use neonates on site, streamlining the entire insect farming process. This paradigm shift presents BSF farmers with novel opportunities. They can now circumvent the complexities and financial burdens associated with breeding, thereby reducing capital and operational expenditures and lessening the need for specialized personnel. Moreover, this decoupling of breeding and rearing processes allows existing BSF farms to utilize external supplies of eggs or neonates for various strategic purposes. These include serving as a backup, expediting the activation of new sites, or avoiding the costs associated with expanding breeding operations. The ability to distribute eggs or neonates to satellite locations or smaller sites, where establishing separate breeding facilities is economically unfeasible, further underscores this flexibility. In conclusion, the separation of breeding and rearing in BSF farming is a critical step towards enhanced efficiency and business viability. It enables companies to concentrate on specific aspects of the production process, aligning with the broader industry trend of specialization and focus.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Influence of feed supplementation with BSFL on the production price of chicken meat

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An environmental life cycle costing (LCC) assessment of organic, slow-growing local chicken breed partially fed on live black soldier fly larvae (BSFL) was conducted. The aim was to look into the difference in and a comparison of economic inputs and outputs between the chicken grown with and without 10% live larvae supplementation. The LCC was developed to ensure a structured and quantitative approach. It was modeled based on the information obtained from the project partners (ERA NET Poultrynsect, PRIMA ADVAGROMED and PRIMA CIPROMED) and the current prices of goods and services available on the market. The model included the 240-chicken farm, rearing regional, organic, slow-growing broilers, which fully corresponds to what was done within the experiment, excluding the scientific activities. The scope of the study included operating expenses and followed the cradle-to-slaughterhouse gate perspective (with further extensions of waste treatments) including live BSFL production, chicken rearing, chicken slaughtering, and packaging. The profit of the bird-rearing company was not included in the analysis. The production price of modelled 1 kg of packed ready-to-cook chicken carcass was €15.436 for birds with BSFL supplementation, and €14.195 for birds without. The highest single contributor to the price was labour, followed by feed. As the inclusion of insects into the chicken diet was done through supplementation to the feed and not substitution, the chicken meat price increased by almost 10%. However, even if compared with the price for 1 kg of organic poultry in Italy, which is €9.90, the price of chicken used in the research is rather high. Thus, to increase the competitiveness of the organic, slow-growing chicken that is partially fed with BSFL, improvement in the efficiency of both the insect and the chicken farm is needed, and can be achieved through significant upscaling. Otherwise, higher competitiveness might be achieved through feed substitution with BSFL (instead of supplementation), or a choice of a chicken breed with a shorter life cycle.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Decision support system for insects production

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Insect value chains are complex systems with non-linear connections among various economic, environmental, and social variables. Industrial stakeholders often require clarity in potential environmental impacts, social issues and economic costs for efficient decision-making. This study employed a modular life cycle approach to analyze insect production chains, considering economic, environmental, and certain social aspects within the frameworks of the EU projects SUSINCHAIN, ADVAGROMED, CIPROMED and GiantLeaps. Integration of results from separate modules was based on developed scaling factors. These factors were further used to design multiple production scenarios, employed for multi-objective optimization (MOO) and the design of a decision-support system (DSS). The DSS accounted for a mix of sustainability relevant factors. The DSS functions as an online tool available in open access and could be further optimized and programmed in future research. Moreover, MOO allows for identifying a larger range of optimal scenarios with varying objectives. For example, for the companies producing *Hermetia illucens* and operating at large scale in Germany the optimal from sustainability perspectives will be 5 feed scenarios: (1) 98.3 % brewery spent grains, 1.3% chicken feed; (2) 81.5 % brewery spent grains, 18.5% vegetable rests; (3) 81.8 % brewery spent grains, 8.4% vegetable rests, 7.3% chicken feed, 2.5% milling rests; (4) 67.4 % brewery spent grains, 14.1% vegetable rests, 18.5% chicken feed; (5) 91.6 % brewery spent grains, 8.4% chicken feed. Further research is needed (and planned) to identify a broader spectrum of optimal scenarios for a range of alternative protein sources as well as to validate MOO results through the real-life development of sustainable insect chains and to assess its 'optimality.'

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

HiGa: *Hermetia illucens* for a green agriculture

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The EU has to import 17 Mt of protein for feed and an estimated 129 Mt of food waste is produced each year. One way to solve these issues is using *Hermetia illucens* (HI). A new project called "*Hermetia illucens* for a green agriculture" (HiGa, 2024-2027) aims to improve the economic viability of insect farming, increase the protein self-sufficiency and reducing the need of chemical fertilizers. Specifically this will be done via: (1) Rearing HI: Currently, the industry works with an (near) wild fly. Artificial selection will vastly improve the size, feed efficiency and nutritional quality. Furthermore, farming practices will be further tailored to the larvae to boost production efficiency. (2) HI as feed: The HI protein meal digestibility (85%) in aquaculture will be increased and optimized using enzymes. The potential of the fat fraction as ruminant feed is also assessed as it may reduce the enteric methane production due to the particular composition. (3) HI frass: Frass has the potential to replace chemical fertilizers. Yet, the composition of frass is very variable. Synergies between frass and other currently used soil amendments/fertilizers (e.g. other manure types) will be assessed. (4) HI emissions: quantifying and reducing GHG and ammonia emissions throughout the value chain by: varying feed and rearing methods, reducing methane emissions and reducing loss of N and C of frass. (5) HI sustainability: an environmental and economic impact assessment will be made ensuring a bright future for the farmers. It is expected that by the end of the project, HiGa will result in a more productive, greener, sustainable agro-economy, reducing our protein deficiency and mineral fertilizer dependency. HiGa is financially supported by the Green ERA-Hub.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Insect farming as a transforming model of sustainable tourism village based on circular economy

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This study aims to examine the practice in insect farming as the central of creating sustainable tourism village. World best tourist destination such as Bali is facing waste problem which mainly due to the increase of visitors. Bona village in Bali is selected as the potential location for the transforming model as it just enacted a source-based waste management program and built facility called "TPS3R" – Landfill for Reduce, Reuse, Recycle. Although TPS3R provides opportunity for the village to upcycle the organic waste i.e. composting, its low value made the continuity of business model is in question. Black soldier fly (BSF) insect farming along with the application of circular economy principles in tourism may come with a viable solution in the long run. The study used a qualitative method, whereby the data were collected through focus group discussion and in-depth interviews with key persons of the village. The analysis showed that BSF insect farming can be the factor for creating successful sustainable tourism village that utilize a circular economy. The model focuses on circularity in engineered BSF production system into tourism value chain that support economic, social, and environmental sustainability. BSF technology emphasizes ways to develop and advance village's waste management program of food so that it never becomes waste then bringing landfill waste as close to zero as possible. At the same time, economically the selling product of BSF live larvae accounted at least 5 times higher in value compared to compost. Involvement BSF products in nature tourism like farming activities leave a positive footprint for the tourists through awareness of regenerative food system. Packaged by cultural tourism in traditional arts not only being able to complete the whole experience but also increase the welfare of local communities. The model also performs as a supplement to UNWTO's global roadmap for food waste reduction in the tourism sector which absent to consider insect farming as a powerful tool to achieve target in 12.3 of the SDGs. The study represents a unique contribution of insect industry by promoting innovation, differentiation, and diversification of income streams in tourism sector to embrace a resilient pathway as well as solving various waste problems.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Development of a regionalized dynamic weighting method for the environmental impact of insect protein sources

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Our innovative approach characterizes environmental impact weighting based on total or specific production impacts at the country level, facilitating relevance assessment. We have developed a methodology to calculate weights for insect proteins like crickets, mealworms, and black soldier flies, integrating their country-level eco-potential linked to environmental impacts. This method addresses challenges in existing methodologies, including policy changes, contextual adaptation, method specificity, and intangible values. Relative impact weights, normalized by arable land and population, cover greenhouse gas emissions, land use, water use, and energy use. The dynamic eco-potential is influenced by evolving country-level per capita impacts, affecting food product impacts. Comparisons with literature-based LCAs highlight alignments between absolute emissions and relative impact weightings. Additionally, we've developed a parallel methodology to calculate eco-potential points for selected insect proteins based on protein supply in countries. Results show a dynamic eco-potential influenced by evolving country-level per capita impacts that affect food product impacts. Combining the Country Weighted Impact (CWI) from four indicators provides combined eco-potential values for selected insect proteins. Higher country-weighted impact for a specific insect protein in an indicator represents a better production option in a specific country with a lower relative impact weight. Absolute impacts may be higher in a given country, but its country-weighted impact could be lower. Normalized results do not imply an additional burden due to a country's existing environmental impacts. Environmental impacts from insect production may have a small impact but could add to the burden per person's available land, necessitating more land with additional production. Considering both absolute and relative environmental sustainability or eco-potentiality can contribute to a more sustainable food system.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

How much does it cost to setup an industrial insect farm?

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Insect companies have collectively raised capital in excess of US $1.3 billion to build industrial insect farming facilities throughout the last decade. With the first generations of commercial insect farms completed and in operation, there's yet remarkably little published actual data about how much it costs to setup an insect farm and how much it *should* cost to set up an insect farm for future investors. Motivated by the 'Iron Law of Mega Projects' as postulated by Bent Flyvbjerg ("Over budget, over time, over and over again."), we designed a survey capturing quantitative and qualitative elements of project development (based on PMP framework) with respect to insect farms. The objective was to investigate what a reference class for insect farming projects may look like and to crystallize early collective learnings with regards to the successful scoping and delivery of insect farming projects. The results of the industry survey shared among more than 20 leading insect companies around the world provides a starting point of a reference class framework for insect farming projects and answer to the question 'How much does it cost to setup an industrial insect farm'. Responses suggest that CAPEX plans range between US $2-4 million per 1,000 tons per year of production capacity of insect protein meal. It also indicates a fat tail distribution in terms of both cost and timelines for project delivery. As many facilities have only recently entered regular operations, the data for actual capacity vs planned capacity is still preliminary. We conclude and propose for annual updates to this initial data set to help the industry and stakeholders build a more quantitatively informed benchmark that leads to increasingly realistic project plans, avoids costly disappointments and helps the industry deliver on its financial and sustainability promises.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Putting a number on insect welfare! How technology can help. Presenting results from Insect School case study, Part 2

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Directly connected to this abstract is part 1, which focuses how we can put animal welfare into practice. Freedom for hunger or thirst, freedom for discomfort, freedom for pain and disease, freedom to express normal behaviour and last freedom for fear and distress. Much of this can be quantified, registered through technology and scored. Leaving us with a measure of welfare. This so called part 2 showcases a practical case study done at the Insect School wherefrom results are presented. The tests focussed on physiological, behavioural and environmental conditions, carried out with the black soldier fly larvae. Test were carried out on pilot scale, with substrate volumes up to 2,500 kg to get a representative view how this would be in full production. This was not a laboratory test. Two objectives. Firstly, we sought to define and quantify the current state of the black soldier fly industry. This involved examining the present five freedoms of BSF explained in part 1. Secondly, we aimed to explore ways to enhance these. The presentation will comprehensively showcase the results from each situation, elucidating our animal welfare score and the extent to which we utilize data to assign a numerical value to BSF welfare. The key question. To not only demonstrate but also proactively continue to prove that ensuring animal welfare in insect farming is a standard practice. Moreover, the presentation will delve into the implications of animal welfare on the cost price of black soldier fly end products. The ultimate goal is to determine whether it's feasible to maintain an excellent and consistently proven level of animal welfare while remaining financially attractive. Finally, we want to conclude if we can improve the 5 freedoms and create a new standard. To ultimately 'put a number on sustainability'.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Optimising environmental benefits of insects ingredients: identification and optimisation of main production factors

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All over the world, agriculture and agri-food industries inevitably generate losses. Bioconversion consists of recovering food-grade materials that are destined for destruction. Black soldier fly (BSF, *Hermetia illucens*) can valorize various food waste and can thus be produced all over the world in a circular economy logic. This solution responds to the growing need for sustainable ingredients and particularly proteins for animal nutrition (aquaculture, poultry, pigs, pets). Over the last decade, insects producers have invested on the development of zootechnical approach and industrialization in order to develop technically and economically efficient models. Products are being commercialized on the feed markets and many new farm projects are being under development. Although Bioconversion by insects is a virtuous and natural-like process, evaluation of environmental impact and benefits highlighting are key elements for sustaining long term development of insect sector. Several tools can be used, such as Life Cycle Assessment (LCA), carbon footprints evaluations and avoided greenhouse gas emissions. MUTATEC has built its first industrial farm in 2020 and thus has cumulated data (energy, water, etc.) over two years of production. Using then, projections on bigger farms over a several years period have been evaluated too. The conducted carbon footprints and LCA evaluations were based practical experience and economic allocations; both on raw materials to feed the insects and on the insects related products. The results highlight the key-role of four main parameters: the substrate types, the energy source, the zootechnical management and processing technologies. The use of raw material such as wheat-bran should be limited. The use of food losses and agri-food residues seem the most promising approach, coupled with efficient heat recycling and limited processing steps. Based on those data, will emerge some desirable related developments.

INSECT ENVIRONMENTAL SUSTAINABILITY AND ECONOMICS

Adapting insect biorefinery for achieving circular economy and sustainable development goals in Taiwan

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Taiwan's commitment to net-zero carbon emissions by 2050 creates a unique opportunity for insect biorefinery to contribute to the circular economy and Sustainable Development Goals (SDGs). A research consortium of insect biorefinery systems has been proposed and implemented, utilizing black soldier fly (BSFL) and yellow mealworm (YML) larvae to yield value-added products while achieving a net-zero emission goal. The process involves two stages: simultaneous biological pretreatment and preservation of agro-industrial organic wastes (such as spent mushroom roots, spent soy pulp, and pineapple peels), as well as insect conversion. The total duration of the process is 30 and 90 days for BSFL and YML, respectively. Following insect bioconversion, a significant quantity of larval biomass is produced, which is then used to create protein feed, chitin, melanin, functional peptides, biolubricant, biochar, and other beneficial products. Regardless of the insect species used, the waste reduction and bioconversion conversion rates are around 60% and 20%, respectively. Despite different stacking space requirements, both insect species exhibit equivalent productivity. To increase the yield of larval biomass, reduce the development time of YML, and accelerate bioconversion, wheat bran is mixed with organic wastes at a recommended percentage of 40%. This approach shortens ten days of development time while increasing the conversion rate by 50%. As such, a straightforward insect biorefinery scheme with a net-zero emission objective has been devised, and the output has been estimated.

INSECT AS TRADITIONAL FOODS, FOOD SECURITY AND CULTURAL PERSPECTIVES

What makes an eatable insect edible for human?

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While there are a million species described by science, and undoubtedly several million on Earth, only around 2,000 species have so far been detected as being consumed or having been consumed by man. What are the characteristics that motivated this choice? By comparing the leading edible species in Latin America and sub-Saharan Africa, we have been able to identify a number of common features that have motivated the selection of species collected and consumed. The size of the edible stage, the time in the annual cycle when the species is available, its abundance and the ease with which it can be collected are essential factors. The fact that the species live on plants that are the object of particular attention, such as palms in Africa and America, or agaves in Central America, is also an important factor in choice. The species' proven or potential toxicity to vertebrates may play a role, but man's ability to detoxify insects through culinary preparation may modify this factor. This analysis sheds light on the traditional use of edible insects. It can also guide us in the objective choice of species likely to be farmed.

INSECT AS TRADITIONAL FOODS, FOOD SECURITY AND CULTURAL PERSPECTIVES

Edible insects and sustainable food security: a nutritional analysis of traditional diets

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Undernutrition is prevalent and rising in sub-Saharan Africa, where rising temperature and population threaten food security. Edible insects can help combat this; they are cheaper and less environmentally damaging to raise than alternative protein sources. However, knowledge on insect nutritional content, key in selecting species for rearing, lacks consensus. We present and analyse nutritional profiles of five edible insect species, combining original profiles from individuals sampled in Ghana (3 per species) and literature profiles from meta-analysis. Three of these – the African palm weevil (PW), African termite (T), and shea tree caterpillar (STC) – feature heavily in traditional diets, while two – the black soldier fly (BSF) and house fly (HSF) – are industrially raised in the global North. After inactivation, freeze drying, and milling, individuals were analysed for proximate, fatty and amino acid content following standard procedure. Species showed nutritional differences, with the traditionally consumed species often performing best. The STC showed the highest protein content (~60% d.m) and the PW by far the highest fat content (~55%). These two species also showed the highest content of poly-unsaturated (~35% of total fatty acids) and mono-unsaturated (~45%) fatty acids respectively, which are important in development. All species showed amino acid profiles to meet human requirements. Results promote insect use in human diets. They also suggest research effort, often restricted to species of commercial interest, should be expanded to profile nutritional diversity in traditional diets. With that in mind, we outline future work resolving nutritional variation in PW populations across Africa and genetic diversity underpinning this. This species shows nutritional potential, can circularise palm agro-systems, and so is a front-runner in promoting sustainable food security across Africa.

INSECT AS TRADITIONAL FOODS, FOOD SECURITY AND CULTURAL PERSPECTIVES

Entomophagy culture in north-west Ghana: opportunities and challenges for food security and sustainability

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We aim to improve protein intake in Ghanaian households, particularly for children, by ensuring a steady supply of protein. A cross-sectional survey was conducted using a semi-structured questionnaire administered purposively to adults in the Upper West Region of Ghana to explore the types of edible insects that are consumed as alternative sources of protein and analyse factors influencing entomophagy. The findings were presented in the JICHA (Japan International Child Health Association) Journal proceedings. The results showed that people in the region consumed six types of insects (winged termites, shea tree caterpillars, palm weevil, locust, crickets, bee larvae) and one arachnid. The 7-point Likert scale ratings revealed that 'convenience', 'naturalness', 'cultural acceptability', 'experience', 'higher nutrients', 'medicinal properties' and 'pleasure of eating insects' influenced entomophagy. Also, regression analysis showed that age and gender were significant ($P < 0.05$) determinants for accepting insects as food. Major negative impressions were 'insect food is strange to eat', 'insect food is for the poor' and 'insect food is 'poisonous'. This study highlighted the importance of preserving existing entomophagy cultures, conserving genetic resources, and addressing responses and challenges to changing dietary habits in the future, as well as helping address Sustainable Development Goals (SDGs): (1) No poverty, (2) Zero hunger and (3) Good health and well-being.

INSECT AS TRADITIONAL FOODS, FOOD SECURITY AND CULTURAL PERSPECTIVES

Assessing halal certification of insects

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In 2022, the Food and Agriculture Organization of the United Nations (FAO) released a publication titled 'Thinking about the Future of Food Safety – A Foresight Report', which notably classified insects as new food sources. This classification was prompted by the growing interest in incorporating insect-based products into the wider consumer base, particularly in regions where insect consumption is generally unpopular. Consequently, this burgeoning interest raises the pivotal question of whether products derived from edible insects can obtain halal certification for Muslim consumption. In response to a fatwa by the Singapore Fatwa Committee on alternative proteins sourced from insects in the recent year, the Islamic Religious Council of Singapore (Muis), the religious authority responsible for regulating halal certification in Singapore, is considering the potential halal certification of food products derived from insects. This presentation aims to illuminate the rationale behind the aforementioned fatwa and delve into the certification requirements that must be fulfilled for the halal certification of insect-derived food.

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