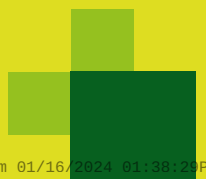




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Foreword

'Insects to Feed the World' was born from a United Nations Food and Agriculture Organization (FAO) initiative. The conference was first organized in the Netherlands, through the collaboration of the Wageningen University and Research Centre (WUR) and the FAO. The conference was also held in Wuhan, China in 2018, and was organized as a virtual event from Quebec, Canada in 2020.

The 4th edition of 'Insects to Feed the World' from the 12th to the 16th of June 2022 in Quebec City (<https://ifw2022.org/en>) has continued its mission to facilitate the dialogue and the exchange of research results on the use of insects in the context of food and feed security, toward building evidence-based legislation, advancing communal knowledge, and promoting innovation for the sector. The conference gathered academics and experts from around the world to exchange on the latest advancements in the field of edible insects and entotechnologies.

IFW2022 was a unique opportunity to propel edible insects as an integral part in the future of sustainable agriculture and food systems. A part from a diversified scientific program, several panels were held to establish first contact and identify opportunities between academics and the industry (*Speed Networking activity*); to discuss about the wide range of applications of insect product commercialization for insect farmers (*Frass to Feed the World*); how to support insect farmers with critical equipment, software, and engineering support to scale up insect production and maximize output (*Scaling up insect production with key technologies*) and how to tackle both insect and worker health in insect farms (*Health & biosecurity workshop*). The second 'Global Roundtable for Insect Agriculture' initiated in Wuhan in 2018 allowed the international industry associations to continue their discussions on common issues.

The conference also sought to provide training on how getting started in insect rearing on a small to medium scale (*Training workshop*); to showcase the activities of the local industry (*Technical visit at Montréal*) and reach a wider audience in organizing 'Eating Insect North' at Le Grand Marché de Québec, a public event exhibiting local producers and transformers in edible insects across multiple kiosks, learn through conferences for the general public and view culinary demonstrations (<https://ifw2020.org/en/program/eating-insects-north>).

Finally, the conference has sought to begin initiatives to harmonize fundamental research procedures that will assist the sector to evolve in a more coordinated manner and contribute to coherent development of related policies and regulations (*OECD Workshop to establish standard methodologies for R&D*); to create in the following years new course training, transfer technology activities and research at an agronomic level (*Launch of the Chaire de Leadership en Enseignement en production et transformation primaire d'Insectes Comestibles (CLEIC) at Université Laval*) (<https://cleic.fsaa.ulaval.ca>) as well as to establish the foundation for the establishment of a new interdisciplinary scientific society that draws together members from diverse scientific backgrounds under the unified discipline of insects as food and feed.

The pooling of academic knowledge, in partnership with industry, will be a guarantee to maintain the impressive advancements required to anchor edible insects as the food and feed of the future. See you soon at IFW2024!

Marie-Hélène Deschamps and Grant Vandenberg

Keynotes

Edible insects: progress and prospects

A. Van Huis

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The literature that has appeared over the last few years on edible insects is reviewed and an outlook on the future is provided. In the last decade attention has shifted from harvesting insects in the tropics to farming insects all over the world for food and feed applications. Most production now is targeted towards pet food, but it will shift to aquafeed in the next two decades. More than 80% of all scientific publications dealing with edible insects appeared during the last five years. Crickets and mealworms are used for human food while the black soldier fly is the main species used as animal feed. The criteria for choosing an insect species are based on the possibilities to automate, use cheap substrates available in large quantities, shift to other similar species with low susceptibility to disease, and market the produce. Selection for high-performing strains is increasingly applied to improve production. The environmental impact of insect production compares well to other alternative proteins. In addition, insects can degrade many types of organic waste streams, supported by microorganisms in the gut or in the substrate. Edible insects are not only a good source of nutrients but they also provide health benefits for humans and animals, as well as for plants (leftover substrate). The health effects are brought about by chitin, antimicrobial peptides and specific fatty acids which increase disease resistance. An overview is given of the possibilities to use insects as feed for pets and as ingredient in pig, chicken and aquafeed to (partially) replace soy. The challenge of overcoming food neophobia and disgust for Western consumers is reviewed. Is the argument that edible insects are more sustainable than meat convincing enough for consumers? Should processing techniques be simple or more elaborate by separating proteins, fats and chitin, or by using fermentation? When insects are reared on not legally authorised waste streams, other industrial applications then food and feed are possible. The sector is developing fast thanks also to an increasingly conducive legislative framework. But will there be room for small insect farms, considering the rapidly expanding big companies? More (inter)national collaboration will promote innovation.

Science for peace: socio-economic impact of organic waste conversion by black soldier fly larvae in post-conflict areas

K.B. Barragán-Fonseca

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Science should respond to the social reality, helping to resolve the problems faced by humanity. All areas of science are capable of contributing to resolving social problems; that is, they all have an idea, a species or a process that has the potential to be disruptive innovators to resolve a problem or to support a community. Insects have the potential to transform societies. Insects like black soldier flies may be produced locally on organic waste, providing protein as well as other nutrients for animal feed, thereby reducing dependence on high-cost imported protein sources and contributing to a circular economy. Indeed, insects may be used to replace conventional sources of protein in animal feed to improve socio-economic conditions of small farmers in general and mitigate gender inequality, as has occurred in Kenya. In Colombia, a country currently in a post-conflict stage after over 60 years of war, we were able to help resolve a community problem: the high cost of fish feed through implementing a small pilot plant for production of black soldier fly larvae to feed fish, reducing the costs up to 23%. This project of implementing the use of insects as feed by communities of ex-combatants and others affected by the armed conflict is called Insects for Peace, and is an example of how black soldier flies could help reintegrate ex-combatants into the local economy and thus to contribute to peace through science. This project has shown that even when large-scale transformations are not possible, small-scale initiatives can make significant contributions. Societal transformations – such as from war to peace – unleash social, economic, and political challenges that need to be dealt with by many committed actors through multi-, inter-, and trans-disciplinary perspectives. Insects for Peace has been made possible through the collaborative efforts of scientists of a variety of disciplines as well as personnel from independent organisations leading to the support of post-conflict transformation in Colombia.

Environmental impact of insect production for food, feed and beyond

S. Smetana

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Mass production of insects is calling for environmentally optimised, economically efficient and socially accepted insect value chains. It is a complex task considering a great variety in insect species, production scales, feed formulations, social preferences, etc. Performing the study on the sustainability assessment in the scope of SUSINCHAIN project (funded by EU) it is necessary to draw the conclusions for the optimal production chains from multiple objectives. The studies relied on modular life cycle assessment (LCA) approaches for environmental impact, analysis of cost structure of production and inclusion of specific social factors (e.g. fair wage potential). Research is based both on continuous review of published studies, but also on the modelling of first quality data received from insect production and processing companies. First, the study identified feasible approaches for the modelling of insect production chains, which can be used for the facilitation of comparability of LCA and sustainability studies in the conditions of limited data. Feed production and nutritional quality, duration of insect cultivation and level (and type) of final processing were the main environmental hotspots, which defined the environmental efficiency of insects. Further, multi-objective optimisation included the scale and geography of production, species and type of final product, reference (functional) unit, type and price of feed as well as local social conditions in the analysis of sustainable insect chains.

The alternative protein landscape: promises and challenges faced transitioning from start-up to scale-up

M. Ashour

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Industries around the world have been radically re-shaped and impacted because of the effects of COVID-19, and the insect agriculture industry is no different. The past two years have seen consolidation, decline, growth, and transformation within our industry. At present, there are nearly a dozen companies around the world, including Aspire, engaged in significant commercial projects, putting the collective promise of our industry – namely, that insects have the potential to be a cost- and environmentally-effective solution to intractable problems of proteins supply – to the ultimate test as we transition from start-up to scale-up. In this keynote presentation, the author will elucidate the challenge faced by the industry in the coming years of maturation even as the world rebounds from the pandemic, along with the opportunities the industry has to secure a leading position in the alternative protein landscape.

Genotype-phenotype correlations in optimising biology: lessons learned from other domesticated systems

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Domestication of plants and animals has long been a process of specific breeding, and only recently (~50 years) have genetics of these organisms played a role. Insects as food and feed present a unique opportunity to start with a head start for which we can take the lessons learned from other successful systems and apply it to insects that have an environmental impact on the food chain. We'll discuss these lessons, and how they can be applied to generate a more robust agricultural system to feed the world.

Safe, legal and sustainable – a European perspective on upscaling insect farming

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The insect sector – a new agricultural industry that is gradually growing into a reputable actor in terms of products' quality and sustainability – has the potential to become an important agri-food player, but also a strategic link of global food and feed chains. Notably, insects have been recognised for their role in complementing both humans' and animals' diets, having an enormous potential to improve the circularity of the agri-food nexus. The benefits of insect farming go beyond that – insect farming brings local solutions to farmers, improving circularity in crop and animal farming while also reconnecting regional agricultural supply chains. Therefore, as recognised by the Food Systems Summit held by the United Nations in 2021, such new sectors can play a relevant role in accelerating progress on the targets of the UN Sustainable Development Goals (SDGs). Since the establishment of the International Platform of Insects as Food and Feed (IPIFF), the priorities of the European insect sector revolved around unlocking the opportunities under the European Union (EU) legislation. Following the achievement of several regulatory targets in 2021, the natural question is 'What follows next?'. This presentation will provide an overview of the European vision for upscaling insect farming by 2030, a strategy that focuses on three key pillars: (1) advocacy and communication; (2) further unlocking regulatory opportunities; and lastly (3) quantitative and qualitative growth. Most importantly, the speaker will highlight key trends in the field of insects as food and feed, as well as their relevance in a European and global context.

Phenotypic exploration is the tip of the iceberg: fraud, espionage, intellectual property, contamination, & legality

J.K. Tomberlin¹, C. Picard², H. Jordan³ and C. Peyer⁴

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Great strides have been made in establishing the insects as food and feed industry. Recent advancements demonstrate the breadth of abilities each of insect species mass produced. In fact, many facilities have developed their own strains of insect that is used in their processes. The purpose of this presentation is to describe the phenotypes across insect models that have been developed and opine on those that could potentially developed. These examples will be bridged with topics of interest across the spectrum of the industry that includes fraud, espionage, intellectual property, contamination, and legality.

Insect as food & feed/processing, conservation & food safety of insect products

Fate of polychlorobiphenyls in the insect *Tenebrio molitor*: consequences for further use as food and feed

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There is a growing demand for animal protein while the resources used for animal feeding are limited or may raise environmental issues. Thus, there is a need to find alternative and sustainable protein sources. Edible insects may be an interesting solution with a good nutritional value. Learning from the BSE prion crisis, it is essential to ensure the safety of insect products and the chemical contamination is a crucial issue. Among priority contaminants, persistent organic pollutants (POPs) such as polychlorobiphenyls (PCBs), dioxins (PCDD/Fs) or polycyclic aromatic hydrocarbon (PAHs) are of serious concern in food supply. In this context, this study explored the ability of yellow mealworm (*Tenebrio molitor*), the most widely bred and traded insect species in Europe, to bioaccumulate PCBs from their feeding substrate. *Tenebrio molitor* larvae were reared during 20 days in a temperature and humidity-controlled incubator and fed with wheat bran artificially contaminated with PCBs at a concentration of 0.67, 4 or 24.4 µg/kg (n=9). The larvae PCB content was then measured based on an analysis by gas chromatography hyphenated with a micro-Electron Capture Detector (GC-µECD). Whatever the PCB contamination level, no significant impact was observed on the larval growth. The bioaccumulation factors (BAF = concentration of PCBs in larvae / concentration of PCBs in wheat bran) obtained with dried larvae, the most common commercially available form, ranged between 1.5 and 3. This study highlights the ability of *Tenebrio molitor* to bio-accumulate PCBs from their diet during rearing and the significant impact of the drying process on the bioaccumulation factors. This demonstrates the importance of considering the quality of the substrates used for farming insects as food and feed in terms of content in chemical contaminants including POPs like PCBs.

Nutritional, functional, thermal characteristics and estimated shelf-life of defatted *Rhynchophorus phoenicis* larvae flour

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Rhynchophorus phoenicis is an insect of the coleopteran family. They are widely consumed in Africa and particularly in Cameroon. These larvae are rich in lipids and proteins. However, they are very perishable and their use as food ingredients remains limited due to their high fat and water content. The present work aims to produce low-lipid content larva flours of *R. phoenicis* and to determine their nutritional, functional, thermal properties and their estimated shelf life. The flour was obtained by the cooking-pressing process. Thermal properties were determined by thermogravimetric analysis and differential scanning calorimetry. The shelf life of the flour was estimated using adsorption isotherms. The results obtained show that the flours have low lipids (8 g/100 g of flour) and high proteins content (69 g/100 g of flour). Their water and oil absorption capacities (1.90±0.02 ml/g), (2.58±0.01 ml/g), swelling power (3.83±0.01 g/g of flour), foaming properties (5.84±0.03%) and foam stability (67.78±0.03%) make them good ingredients for food formulations. Thermal analysis revealed a small loss of mass (5.8%) at the beginning of treatment and the formation of a residue at 600 °C corresponding to 5% of the initial flour mass. Differential scanning calorimetry showed a glass transition temperature from 10.03 to 11.14 °C as well as two melting points at 19 and 22 °C and one crystallisation point (20 °C). Regarding the storage capacity, the adsorption isotherms obtained can be modelled by the Guggenheim-Anderson-and-de-Boer (GAB) and Brunauer-Emmett-and-Teller (BET) equations. The shelf life is estimated at 300 days when flour is packed in polyethylene and stored at 20 °C in enclosure with a relative humidity of 90%. In short, the cooking-pressing process makes it possible to obtain flours of high nutritional and technological values. The resulting flours can be used as ingredients to fortify foods with poor proteins content.

Industrial demonstration of an enzymatic treatment to improve protein quality in insect meal from *Hermetia illucens*

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The aim of this work is to develop and optimise an aqueous enzymatic pre-treatment of fresh larvae of *Hermetia illucens* at industrial scale followed by centrifugation to reduce the fat content in insect meals and enhance the protein quality, work developed in the European Project SUStainable INsect CHAIN (SUSINCHAIN). Firstly, conditions were established at lab scale. Three fractions were obtained: (1) floating oil fraction, (2) aqueous fraction (mainly soluble proteins and hydrolysed proteins), (3) solid fraction (mainly chitin and insoluble proteins). The effects of different proteases were screened, and protease (Prot1) was selected. Next, conditions were optimised through experimental designs combined with response surface methodology (RSM) to optimise the fat extraction by applying Prot1 and lipases (Lip1, Lip2) with different enzyme doses, and reaction time. This resulted in the following optimal conditions: 0.2% Prot1, 0.75% Lip1, 0.75% Lip2 at acid pH and mid temperature under agitation for 4 h followed by thermal treatment at 90 °C for 2 h and finally, centrifugation. This process was validated at lab scale obtaining insect meals with protein content of about 38% dw and 20% dw residual fat, representing 52.5% fat extraction yield. The total amino acid content obtained was about 30% dw being the most abundant aspartic acid, glutamic acid, and alanine, similarly to the fresh larvae amino acid profile. The *in vitro* dry matter and protein digestibility in the insect meals obtained were approximately 87% dw. These optimum conditions were selected for further validation at industrial scale using a 100 l reactor and an industrial tricanter centrifuge. From the scaled-up process, it was obtained an enzymatically treated insect meal with improved *in vitro* digestibility by over 20% compared to the conventional process without enzymes. When fractionated, the aqueous fraction reached 100% protein digestibility and the solid fraction around 91%. In conclusion, the aqueous enzymatic treatment followed by tricanter centrifugation is a promising technology to reduce the fat content and enhance the *in vitro* protein digestibility.

Application of green processes and emerging technologies for the valorisation of mealworms in a biorefinery concept

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With the world's population predicted to reach 9 billion by 2050, it is essential to find new sustainable agricultural practices and protein sources for the feed and food sectors. Edible insects are a promising alternative biomass that can meet the growing demand for proteins and their derivatives, presenting many advantages over conventional farming, such as: minimal resource needs (green spaces, water and energy), low environmental impact (less GHG and NH₃ emissions), low feed conversion ratio (around 1.7 for insects compared with 10 for cattle) and above all their integration into a logic of circular economy. This research work is part of the RAFINSECT project funded by the HDF region (France) and aims to set up an insect biorefinery. The objective is to fractionate and valorise all biomass fractions such as proteins, lipids and chitin by incorporating them into a variety of food and feed matrices, and into cosmetic products and packaging as well. To successfully establish an insect biorefinery, many scientific and technological barriers must be overcome. Among these are the identification of the best technological pathways for preserving the quality of different fractions while remaining environmentally friendly and energy efficient. To achieve these goals, we are focusing on the use of emerging technologies (instant controlled pressure drop (DIC), pulsed electric field, ultrasound and supercritical CO₂) in order to intensify the transfer phenomena throughout the various transformation stages. In this context, mealworms (*T. molitor* larvae) were pretreated with DIC and PEF, then delipidated and dehydrated by pressing and/or drying. The impacts of pretreatments on larval mortality, pressability, cell structure, drying kinetics and product quality were studied. The processes were compared and optimised. The obtained results confirmed the positive influence of pretreatments used to accelerate the transfer kinetics during the fractionation, extraction and drying operations while preserving product quality.

Impact of the defatting and alkaline extraction steps on the physicochemical properties of house cricket proteins

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House cricket (*Acheta domesticus*), largely reared for human consumption in Canada, represents an interesting source of alternative proteins in the development of insect-based food products. However, the defatting and protein extraction processes could have a major impact on protein structure, hence on their techno-functionalities. Therefore, it is essential to understand how each processing step affects the cricket protein profiles and structures. Frozen adult house crickets were freeze-dried, and cricket powder was either not defatted (ND) or defatted using hexane (HD) or ethanol (ED). Proteins were extracted from both non defatted and defatted powders by alkaline solubilisation (pH 10.5, 40 °C) for 1, 2 or 3 h. The proximate composition (dry matter, protein, lipid, and ash contents) and the colour of cricket protein concentrates were analysed. The impact of each extraction step on protein profiles was observed by gel electrophoresis under native, non-reducing, and reducing conditions. Changes in surface hydrophobicity of cricket proteins were assessed by fluorescence spectroscopy, and free and total sulfhydryl groups were measured by Ellman's method. The solubility of cricket proteins was measured at pH 7. Preliminary results showed that, compared to ND, the defatting step using hexane reduced the protein recovery yield obtained after 1 h alkaline extraction. However, this difference was decreased when the extraction step was extended to 3 h. Protein purity was higher when cricket protein extracts were produced from HD, compared to ND. No difference in the protein profiles of ND and HD protein extracts were observed by gel electrophoresis when alkaline extraction was performed for 3 h. Although defatting is crucial to generate cricket-based ingredients with high protein content and purity, it appears that this step had a major impact on protein recovery yield following their extraction by alkaline solubilisation. Experiments are currently under development to evaluate the effect of the defatting and alkaline solubilisation steps on the modification of cricket protein structures.

Optimisation of industrial scale dielectric drying for stabilisation of black soldier fly larvae (*Hermetia illucens*)

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Drying is one of the most frequently applied technologies to stabilise insects in order to store, transport or process them for all types of applications. While hot air drying and freeze drying are currently applied on large scale in the insect industry, more modern techniques such as dielectric drying are also being investigated. Microwave (MW) drying and radio frequency (RF) drying are dielectric drying techniques already successfully introduced in other industries. For insects, however, the most optimal conditions to obtain high-quality and stable dried products still have to be determined for large scale application. Within the Horizon 2020 SUSINCHAIN project, we investigated the optimisation of industrial MW and RF drying for black soldier fly (BSF) larvae to obtain chemically and microbiologically stable dried products. Microbiological stability is typically obtained when water activity (a_w) reaches a value below 0.60. By varying process conditions such as drying time, insect layer thickness and energy input, the optimal process parameters to achieve this a_w value were determined. For MW drying (MEAM Dry 32), a stable product could be obtained by drying for 15 min at 22 kW power input for 10 kg of larvae. A layer thickness of 1.5 cm was applied while maintaining surface temperature below 85 °C. Moisture content was reduced below 8% to reach a_w values below 0.60. For RF drying (prototype installation, Dymotec), different parameters should be employed to reduce a_w below 0.60. E.g. a layer thickness of 4.5 cm and a core temperature of 100 °C were applied, while using a lower energy input (± 9 kW for 10 kg larvae). However, process time to obtain final products was higher (± 60 min). In a next step, the impact of the optimal drying processes on nutritional and chemical composition as well as on microbiological quality of the dried BSFL are assessed, in comparison with the commonly used drying techniques.

Insects as a sustainable source of proteins: from raw caterpillars (*Samia ricini*) to protein powder

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There is an increasing demand for edible insects that are safe to consume, easily available, and organoleptically acceptable. This demand requires the need to improve the processing and shelf life stability of insect's products. Therefore, the best way to ensure efficient utilisation of edible insect in food is to optimise the drying and flour processing steps. The obtained protein powders should present good physicochemical properties and chemical composition. We have worked on the drying of gutted and gutted caterpillars (*Samia ricini*). We applied different drying temperature in an oven (laboratory scale) and a freeze drying on the samples of caterpillars. The protein content determined by Dumas analysis (dry basis) is around 53% for gutted samples and 48% for ungutted samples. No aggregation phenomena (electrophoresis) and changes of structure (infrared spectroscopy) were observed as function of the temperature of drying. The solubility of the proteins of the dried caterpillar powder was around 2% at pH 10 and 15% at pH 3 which is very low. The water adsorption capacity of the dried powder was around 3.2 to 4.3 g of water per g of dried powder, and the oil adsorption capacity was around 1.9 to 3.5 g per g of dried powder. There is no significative difference between samples dried at 55 and 84 °C but an effect of gutting step. We have shown that we cannot discriminate the samples as function of the drying temperature (55 or 84 °C), but that the step of gutting or ungutting affect the properties of the powders, in terms of composition and of physicochemical properties (solubility and water holding capacity). This research work is part of the project funded by the humanitarian organisation Action Against Hunger (ACF-France)

Characterisation and modelling of drying of pressed larvae for animal feed

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Insects are sources of protein and lipid and could be the ideal, nutritious and eco-friendly food. The production of food-grade functional proteins and other high-value ingredients requires the development of insect extraction and separation by thermomechanical processes. The process used to produce insect meal or animal feed pellets, especially the drying step, has not been widely explored in the literature. The drying process is a necessary step to stabilise the insects or to standardise them before industrial treatment. However, the drying step is often a real bottleneck for the whole production line. It is therefore essential to optimise the operating conditions of drying in order to achieve a better energy performance without affecting the quality of the final product. The overall objective of this study is: (1) conduct experimental characterisation of the drying of pressed larvae (mealworm larvae) using a thin layer drying; (2) optimise by inverse modelling the heat and mass transfer properties during drying (i.e. the heat transfer by convection and the mass diffusivity inside the pressed larvae); and (3) predict the temperature and the moisture profiles of the drying gas and the feed pressed larvae. This approach centred on the prediction of drying kinetics is essential for the design of drying equipment on an industrial scale. The experimental work is carried out in a cylindrical Pyrex vein crossed by a flow of hot air with continuous monitoring of the weighing throughout the drying period. This characterisation method is much more representative of the drying reality than traditional intermittent weighing methods. The modelling work is based on the resolution of the microscopic equations of heat and mass transfer. These equations are correctly adapted to the behaviour of several types of pressed larvae for a predictive and accurate simulation. The global approach can be easily applied to many other species of insects.

Extraction of protein from caterpillar larvae by mechanical pressing assisted by pulsed electric field treatment

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Satisfying the growing demand for proteins is one of the major challenges for international organisations, private operators and agri-food researchers. Among several possible additional sources of proteins, the insect solution seems to be relevant and credible as a complementary option alongside other conventional ones (fish and soya). The development of an insect industry requires the development of technically reliable and economically viable transformation processes. This research work is carried within the framework of a partnership between AgroParisTech and The University of Technology of Compiègne and aims to set up a viable process to produce a flour rich in proteins from caterpillar larvae. In fact, these larvae are rich in proteins and lipids. The objective of the study was to fractionate the raw material in order to obtain a liquid fraction rich in oil and a solid fraction (flour) rich in proteins. For this purpose, mechanical pressing and/or drying were used for insect dewatering and defatting. In order to enhance the pressing kinetics and to increase the extraction yield, a Pulsed Electric Field (PEF) was applied as pre-treatment to permabilise the membrane cells and therefore enhance the extraction/drying kinetics. Results showed that the application of a very short (10 ms) PEF pre-treatment at $E=600$ V/cm before pressing improves the extraction yield by about 20% and reduces significantly the extraction time. The dry mass of the press cake was increased up to 78% with a proteins content higher than 50%. Moreover, the application of PEF treatment enhanced the drying kinetics and reduced considerably the energy consumption. Pulsed electric fields seems to be very promising technology. In fact the application of the electrical treatment induces cells membranes permeabilisation facilitating thus the extraction and drying step at moderate temperature ($T<60$ °C) to preserve the product quality. The results obtained in this work will be very useful to pave the way for caterpillar larvae transformation in order to produce high-protein flour.

Health-promoting and unobvious properties of edible insects

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Edible insects are known for their nutritional value and are becoming increasingly popular worldwide as an alternative source of protein. Nonetheless, the increased interest in edible insects is fostering the discovery of their other properties and the benefits provided by the consumption thereof. One example is the use of the antioxidant properties of insect-derived hydrolysates obtained during *in vitro* digestion in the prevention of oxidative stress, which contributes to the development of many diseases. The hydrolysates obtained from *G. sigillatus*, *T. molitor*, and *S. gregaria* in this study were able to scavenge free radicals and inhibit the activity of pro-inflammatory enzymes such as lipoxygenase and cyclooxygenase. The hydrolysates obtained from these insect species also inhibited the activity of enzymes involved in the pathogenesis of metabolic syndrome, i.e. the angiotensin-converting enzyme (the highest inhibitory activity was observed for the hydrolysate from *G. sigillatus* IC₅₀ 28.2 µg/ml), α-glucosidase (*G. sigillatus* IC₅₀ 5.89 µg/ml), and lipase (*S. gregaria* IC₅₀ 66.11 µg/ml). Moreover, the heat treatment process has a positive effect on the bioactive properties of the hydrolysates. Simultaneously, the *T. molitor* larvae exhibit an extremely unusual ability to recycle expanded polystyrene, which is useful for protection of the environment. However, it is important to know the effect of consumed polystyrene on these insects. A Styrofoam-feeding test was conducted for 30 days with unlimited access to Styrofoam without another feed, with a control sample fed by bran. The extract from insects fed with foamed polystyrene did not show significant cytotoxic properties; furthermore, the extracts obtained in this way had lower cytotoxic properties than the control ones. Moreover, the mealworms fed with Styrofoam were determined to have higher protein (51.93%) and ash content (4.81%) with reduced fat content (24.05%) than insects receiving the conventional diet (37.06%, 2.82%, and 43.74%, respectively). Additionally, Styrofoam did not influence the health status of gut cells in the examined insects ($P < 0.05$). The results obtained in this work will be very useful to pave the way for unconventional uses of insects.

Development of honey bee (*Apis mellifera*) drone larvae harvesting and processing technology

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Bee (*Apis mellifera*) drone larvae is a high-quality by-product of beekeeping that contains protein. Beekeepers tend to remove bee drone larvae from the hive to control the spread of the Varroa mite, but larval removal is not always done in hygienic way and therefore further processing of the larvae is difficult. Researchers use a variety of techniques to collect bee drone larvae, but it is not clear which method is the best. It is important not to damage the product during harvest, when fungi and mould can multiply rapidly at favourable ambient temperatures. The aim of the work was to harvest samples by different techniques and to assess the chemical and microbiological properties of bee drone larvae. As a result of the research, the chemical composition of bee drone larvae was determined, as well as microbiological contamination was analysed in the freeze dried product during storage. The harvesting, processing and storage of bee drone larvae may be impaired by harmful microorganisms such as *Mucor circinelloides* and *Candida famata*. Different types of fungi species of the genus *Penicillium* caused spoilage in the freeze dried bee drone larval homogenate, if the moisture content exceeded the optimum value. It's more effective to freeze dry intact larvae because they have much larger surface area to be freeze dried than pressed and homogenised larvae. A change in the colour tone of the drone larvae indicates deterioration during harvesting and storing. Further research is needed into the deterioration of bee drone larvae and the growth of microorganisms during harvesting and storage. The suggested technology for harvesting bee drone larvae is similar to harvesting bee bread when the cell combs are frozen with the product and then separated.

Effect of high hydrostatic pressure on mealworm (*Tenebrio molitor*) protein structure

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Edible insects are gaining interest due to their positive attributes related to food security and environment. However, acceptability of whole insects by western consumers is a hindrance for market development. The incorporation of insect proteins in conventional food matrices may increase consumer acceptance. However, the development of insect protein-based products requires fundamental knowledge about the effect of food processing on edible insect components, mainly proteins, and techno-functional properties. High hydrostatic pressure (HHP) is the most utilised nonthermal technology for microbial inactivation. Depending on the severity of process parameters, HHP induced modifications affect protein structures and techno-functional properties. Nevertheless, little data are available regarding the impact of HHP on mealworm proteins structure. In this work, mealworm protein solutions were prepared at a 10% (w/v) and pressure-treated from 70 to 600 MPa during 5 min. Unpressurised mealworm protein samples (0.1 MPa) were used as control. Structural changes of HHP-treated mealworm proteins were evaluated using turbidity, particle size distribution, intrinsic fluorescence, surface hydrophobicity, gel electrophoresis, mass spectrometry and transmission electron microscopy. Results showed that denaturation of mealworm proteins occurred under pressure, mainly at 600 MPa. The formation of protein aggregates, mainly composed of twitchin, α -amylase and hexamerin 2, was also confirmed. Microstructure analysis indicated a potential involvement of actin and myosin in aggregates composition. For the first time, we demonstrated that HHP induced denaturation and aggregation of specific mealworm proteins. Further analyses are necessary to understand the impact of denaturation/aggregation phenomenon on protein techno-functional properties which is crucial to generate food products formulated with insect ingredients.

Efficiency of activated charcoal and sodium bisulphate to reduce dark-colour reaction in *Tenebrio molitor* protein extracts

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The use of non-thermal technologies to limit the impact of conventional heat treatments on edible protein structures and techno-functionalities is particularly interesting. However, without heat treatments, enzymatic browning is not inactivated. Nevertheless, browning coloration can be prevented or reduced by sodium bisulphite (SB) and activated charcoal (AC). The aim of this study was to evaluate the efficiency of SB and AC as anti-browning agents on *Tenebrio molitor* (TM) protein extracts and to study their impact on proteins structures. Extraction of TM proteins was performed with addition or not (control) of SB (1% w/v). AC was added to TM protein extracts (0-3% w/v) in combination or not with heating (50 °C, 1 h). Protein extracts were recovered, and the impact of SB and AC was evaluated using colorimetry, turbidity, particle size distribution, surface hydrophobicity, intrinsic fluorescence, sulfhydryl groups measurement, gel electrophoresis and proteomic. Results showed that SB could prevent enzymatic browning during protein extraction while causing the denaturation/aggregation of TM proteins. Aggregates were mainly composed of twitchin, hexamerin 2 myosin and actin. Discoloration of TM protein extracts was correlated with the concentration of AC. Turbidity was decreased (0.26 ± 0.02 at 3% w/v) compared to SB (0.04 ± 0.02) and control (0.3 ± 0.0). Moreover, and compared to control, similar surface hydrophobicity values were obtained after AC treatments (without heating) whereas an increase (~25%) was obtained after SB treatment along with a maximal decrease at 3% (w/v) (~20%) under heating. AC-heating treatment induced denaturation and aggregation of globular proteins (α -amylase) with the formation of new thiols-disulphide interactions. For the first time, discoloration of TM proteins was documented, and results can be used to enhance the current knowledge regarding protein extraction and purification.

Impact of soybean curd residues co-conversion by black soldier fly larvae assisted by different gut bacteria on biomass

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The attention for black soldier fly larvae (*Hermetia illucens*) (BSFL) as an alternative ingredient for food and feed products is on the rise. To enhance the conversion efficiency of soybean curd residues (SCR) by BSFL assisted by *Bacillus subtilis* BSFL-CL (Group R3) gut bacteria subsequently obtain high-quality insect protein and lipid for human and livestock consumption. We pretreated SCR with *B. subtilis* BSFL-CL gut bacteria (10^8 cfu/ml) and used BSFL for conversion. BSFL assisted by microbes enhanced the co-conversion process of BSFL and reduced the development time of BSFL from 19 days to 15 days. BSFL assisted with *B. subtilis* BSF-CL had significantly higher dry mass reduction (58.0%) than control R5 and R6 (53.6 and 49.1%) and other groups. Bioconversion in Group R3 (6.4%) were enhanced than the control groups R5 and R6 (4.9 and 4.0%). The nutritional profile in BSFL protein (50.5%) and lipid (28.2%) were enhanced in Group R3 as compared to control groups R5 (45.5%) and R6 (25.3 and 22.6%) respectively. FCR (8.9) of BSFL fed with SCR and *B. subtilis* BSF-CL (Group R3) was significantly lower than that of BSFL fed with R5 and R6 (10.8 and 12.2) and other gut bacteria groups. In addition, amino acids and fatty acid spectra developed on Group R3 showed a satisfactory and higher level of all the essential amino acids needed for human consumption when compared with WHO/FAO standards. All heavy metals (Cd, Cu and Cr) and antinutritional factors (tannin, oxalate and phytate) were also found within the safe intake level of human animal food production. Therefore, when *B. subtilis* BSF-CL was assisted with BSFL developed on SCR improved all its performance parameters as well as nutritional profile in BSFL as compared to control groups.

Deep-fried grasshoppers a delicacy in Maiduguri north eastern Nigeria

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As a young child growing up in Sub-Saharan Africa, particularly in Maiduguri, North Eastern part of Nigeria, I followed my parents to our family farm to collect grasshoppers (GH) from our crops in the early hours of the day when the temperature is cooler and the insects are less active and easier to catch. My mother used to prepare the harvested GH into a delicious, crunchy, and tasty product. We eat the fried and spicy GH as part of a meal or as a snack. The act of eating insects (entomophagy) as a sustainable alternative to meat and fish is being promoted around the world. In Maiduguri, the two-striped grasshoppers (*Melanoplus bivittatus*) popularly called 'Fara' in the Hausa language had been consumed for decades as a local delicacy. They provide a cheaper source of protein to the people of Maiduguri as compared to other traditional meat. Grasshoppers being cool blooded are mostly hunted by young men armed with lamps and bags at night or at dawn when they are in season particularly during the 'harmattan' period of the year (between November and March). The GH collectors use cover-all clothing, hand gloves and boots to protect themselves from thorns, thistles, or snake bites. The collection of GH starts at about 20:00 hours and lasts for about 8 hours. At about 4:00 am, the GH collectors return with the harvested GH in bags to the markets where buyers (mostly women) purchase the fresh and live GH in bags. The processing of GH involves removing the wings followed by blanching in hot water for about 10 to 20 minutes and then flavoured with spices, salt, seasoning, and garlic powder. The spiced grasshoppers are dehydrated under the sun and then fried in vegetable oil until they become crispy. The fried GH are usually eaten by dipping the whole insect into a bowl of spicy powdered chili called 'Yaji' in the Hausa language. The business of selling GH has improved the livelihoods of many unemployed people in Maiduguri, Nigeria.

Microbial dynamics and quality changes during modified atmosphere storage of mealworm paste

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Fresh insects in paste form can be conveniently utilised for further preparations and processing at both household and industrial levels. However, due to high moisture content, favourable pH and rich nutrient profile, they are highly perishable and prone to microbial and chemical changes upon storage. In this study, the effects of high hydrostatic pressure (HHP) blanching and modified atmosphere packaging (MAP) on the microbiological and the chemical stability of mealworm (*Tenebrio molitor*) pastes were evaluated. The pH, colour, lipid oxidation and microbial counts were monitored during four weeks of refrigerated (4 °C) storage. HHP treatment (600 MPa, 5 min) resulted in up to 3 log cfu/g reduction in mesophilic aerobic and anaerobic bacterial counts. All microbial populations investigated during storage of the mealworm pastes indicated that HHP treated pastes could be consumed up to (at least) one month of refrigerated storage. Further, *L. monocytogenes*, *Salmonella* and *E. coli* were not detected in any of the samples and lipid oxidation of the samples was minimal. Overall, this study shows that HHP treatment can successfully reduce microbial growth, assuring chemical quality and microbial safety of mealworm pastes. Moreover, MAP coupled with refrigerated storage could preserve quality and safety of HHP treated insect pastes, thus extending their shelf-life.

Assessment of low-energy electron beam (LEEB) treatment of two whole dried insect products

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One of the major barriers for market entry of edible insects and insect-derived products is product safety. Insects have a high and diverse microbial community, mainly influenced by the food and rearing environment. Consequently, reliable post-processing treatment techniques are needed to produce safe insects/insect-derived products. Common treatment techniques such as heating can lower the nutritional value and product quality. In contrast, low-energy electron beam (LEEB) is an emerging gentle non-thermal treatment technique to maintain the product quality and commercial value. However, its potential application as an insect post-processing treatment step has not yet been investigated. To the authors knowledge, this is the first study that evaluated the use of LEEB as a further treatment step for dried black soldier fly larvae (BSFL) and mealworms (MW). BSFL and MW were sourced from two insect producers. Following harvesting, insects were blanched (BSFL: 80 °C, 2 min; MW: 95 °C, 3 min) and dried (BSFL: 80 °C, 27 h; MW: 90 °C, 3.5 h). Insects were then treated with LEEB in triplicate (250 keV, 12 kGy). The effectiveness of the LEEB treatment was evaluated in comparison to a control (without LEEB). Physical (water activity, moisture content) and microbial parameters (aerobic and anaerobic counts/spores) were determined directly after treatment and monthly in a six-month controlled shelf-life study (24 °C, 65% RH). LEEB was effective in reducing numbers of all microbial parameters in MW. Specifically, aerobic and anaerobic counts in MW were both reduced by approximately 4-log₁₀ colony forming units per gram (cfu/g). Likely due to the longer drying duration (27 vs 3 h), LEEB treatment had a lower effect on BSFL microbial numbers, an approximate 1-log₁₀ cfu/g reduction for aerobic and anaerobic counts. In summary, LEEB can be an effective and gentle processing step to ensure product safety within the insect industry.

Insect processing towards protein rich food ingredients

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Insect processing, basic or more extensive, is a requirement for insect availability in the food chain. The insect matrix is specific, and different from other protein sources. The goal of this work is to review the state of the art in literature on insect processing with relation to the insect's matrix, including the resulting properties of protein enriched fractions. Main types of processing studied include heating, drying, and bio-fractionation. Insect processing is aimed at production of (semi-finished) products from insect raw materials with desired technological, functional, and nutritional properties. Procedures used in literature base themselves on existing techniques, used for known protein sources. For bio-fractionation, wet and dry type of fractionation were used to obtain protein concentrates, isolates or hydrolysed protein fractions. This requires protein separation from lipids and chitin. In the insect matrix soluble proteins are present in the haemolymph, insoluble proteins in muscles and cuticle. The techno-functional properties of insect proteins, including solubility, water and oil-holding capacity, emulsifying, and gelling properties, were in the range of those of milk/eggs and legumes, but depend on type and extent of processing, species, and purity. For drying simple to more advanced techniques were studied, the level of heating being important. Heating, used for preservation, causes protein denaturation and lowers protein solubility. It also denatures native proteases and phenoloxidases. Browning by phenoloxidases is often undesired, species dependent, and also linked to iron complexation and auto oxidation. Native proteases affect protein properties and functionality, even after storage at -20 °C. The effect of processing is insect species dependent, but common mechanisms are behind its effect on the resulting properties. The techno-functionality of the protein rich fractions is determined by the type of protein, protein concentration, technology of production, pH, ionic strength, and the matrix. Although traditional processing techniques are used for insects, and progress is made in the scientific field, an integrative approach specifically tailored to the insect's matrix is missing.

Hermetia illucens L. larvae- associated intestinal microbes reduce the transmission risk of pathogens in pig manure

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Black soldier fly (BSF) larvae are considered a promising biological reactor to convert organic waste and alleviate the impact of zoonotic pathogens on the environment. We analysed the effects of BSF larvae on zoonotic pathogen populations in PM, which showed that BSF larvae can significantly reduce associated *Staphylococcus aureus* and *Salmonella* spp. Then, using a sterile BSF larval system, we validated the function of BSF larval intestinal microbiota *in vivo* to suppress pathogens, and lastly, we isolated eight bacterial strains from the BSF larval gut that inhibit *S. aureus*. Results indicate functional microbes are essential for BSF larvae to antagonise zoonotic pathogens. Moreover, the results of the relationship between the intestinal microbiota and zoonotic pathogens showed that *Oblitimonas*, *Paenalcaldigenes*, *Terrisporobacter*, *Clostridium*, *Fastidiosipila*, *Pseudomonas*, *Ignatzschineria*, *Savagea*, *Moheibacter* and *Sphingobacterium* were negatively correlated with *S. aureus*, *Salmonella*, *Shigella* and *Escherichia coli*. Overall, these results suggest that the ability of BSF larvae to inhibit zoonotic pathogens in PM is accomplished primarily by gut-associated microorganisms; strains isolated from the BSF larval gut can be cultured and have the potential for industrial applications.

Design and environmental sustainability of insect-based dairy substitutes

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For future food systems is urgent to define alternatives for environmentally impacting foods like animal-derived products. Dairy products are in the top animal protein sources; they provide important nutrients such as fats, calcium, vitamin D and B12. The study aimed to develop dairy alternatives (milk and butter/margarine) to define potential production routes and their environmental impacts in comparison to traditional products. Life cycle assessment (LCA) was performed for the developed prototypes. Pilot scale trials identified a potential recipe and process to develop an insect-based milk and butter alternatives. The insect milk prototype was composed with *Tenebrio molitor* larvae, water, ascorbic acid, *T. molitor* extracted fat, and sunflower lecithin. Pulsed electric field (PEF) pre-treatment as a gentle physical electroporation method was used to increase the protein content in the prototype milk from 1.2 to 2.8%. For insect-based butter/margarine substitute the recipe included oils and fats from *Hermetia illucens* and *T. molitor*. It was possible to substitute up to 75% of lipids with insect fats without negative effects on spreading abilities and with improvement of product colouring (yellowish colour), which is not achievable without additional colorants in margarine. The impact of insect milk prototype was 0.76 kg CO₂ eq./kg FPCM in global warming potential, the land use was 0.93 m² and the primary energy required 10.55 MJ; most of the burden was caused by feed production. The comparison between standardised bovine milk, showed that insect milk had overall significantly lower environmental impact (307.62 µPt lower than bovine milk). Substitution of 75% of lipids in margarine resulted in a product which had higher environmental impact than conventional margarine, but lower than those reported for butter. Development of new insect-based products and their impact analysis identified that insect-based dairy substitutes had a potential to become a product with low environmental impact. The results could further be improved by selecting a low impact diet and energy sources with lower environmental burden.

Development and characterisation of some instant infant flours with local tubers enriched with termites

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The objective of this study is to determine the factors to be taken into account in the formulation of an instant infant flour meeting the nutritional needs of children from 6 to 12 months, from flours of local starchy foods, namely potatoes, yams (*Dioscorea rotundata*) and white-fleshed sweet potato enriched with termites (*Macrotherme subhyalinus*). For this purpose, the flours obtained from our starchy foods were characterised. The results showed that the flours obtained were very good sources of energy with total sugars contents of 87.8, 89.94 and 92.87 g/100 g MS respectively for potatoes, yams and potatoes. Concerning *D. rotundata* these contents vary from 1.49 to 3.96 g/100 g DM; from 1.69 to 6.42 g/100 g MS for sweet potatoes and from 1.8 to 6.48 g/100 g DM for apples. These protein contents increased with the incorporation of termites into the formulations obtained. In particular, while the flours of our starchy foods are naturally low in lipids (<0.5 g/100 g MS), after incorporation, formulations containing 7 to 8.5% of lipids are obtained with protein contents of 15%. In addition, the study of the water absorption capacity, the solubility index, the swelling power and the degree of gelatinisation after the process of cooking our flours in an autoclave have shown a decrease in these parameters except the water absorption capacity which increased in autoclaved flours compared to native flours. The incorporation of termites is one of the main causes of the behaviours observed because of their high lipid content which would have interacted with diffuse amyloidosis in the medium after cooking and thus formed an amylose-lipid complex responsible for the decrease in potency. These new functional properties make it possible to form slurries with high energy density and less viscous.

Characterisation of black soldier fly larval protein before and after conjugation by the Maillard reaction

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Black soldier fly has been proposed as an alternative protein source sustainable for both food and feed due to its nutritional composition. The functional properties of this protein can be improved by inducing the Maillard reaction (MR) in protein-sugar mixtures. This study focused on the conjugation and characterisation of black soldier fly larvae (BSFL) proteins and conjugates. The defatted BSFL larvae were subjected to protein extraction, at an alkaline pH. The protein extract was then conjugated with glucose (2:1 w/w, pH 9), incubated at 50, 70 and 90 °C for 30, 60, 90, and 120 min. The products obtained were then characterised and compared. The changes were confirmed by UATR-FTIR, TGA and DSC. UATR-FTIR combined with principal component monitored the protein-sugar conjugates, to show the structural difference among heated proteins and conjugates. The protein extract from the larvae had a high protein content ($67 \pm 0.78\%$) and good essential amino acids in sufficient quantities to meet human nutritional requirements. The heating treatments resulted in the unfolding and reduction of the protein molecule aggregation. Compared with native protein, conjugates rendered low intensity on the amide regions, according to FTIR. The conjugates showed a higher endothermic transition when compared with non-heated proteins. The increased T-max (the temperature at which decomposition is completed) demonstrated that the conjugation of the protein with glucose improved the thermal stability, remarkably. These results suggested that MR with glucose can be a promising way to improve the thermal properties of BSFL protein.

Biochemical evaluation of the black soldier fly larvae and blow fly larvae harvested from poultry manure in Abeokuta

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Black soldier fly larvae (*Hermetia illucens* L.) are notable insect species that have been reported to have significant importance in nutrient recycling of waste and also, as an essential ingredient in animal feed. This study was carried out to evaluate the biochemical composition of the black soldier fly larvae and blow fly maggots naturally occurring in poultry waste in Abeokuta. The proximate composition of larvae samples were determined using Association of Analytical Chemists methods. Amino acids profile was determined using high performance liquid chromatography. Vitamins (A, B, C, D, E and K) were analysed by Spectrophotometric methods and gut digestive enzymes activities (lipase, proteinease, amylase) was assessed by standard methods. Minerals concentration (Ca, Na, Fe, Zn, P, Mn, and Mg) were analysed by the Flame photometry and Atomic Absorption Spectrophotometry. Fatty acids profile was done using the gas chromatography. Data obtained were subjected to one way analysis of variance (ANOVA). The crude protein, fat content, carbohydrate and energy values of the 6th instar of the black soldier fly were significantly higher ($P < 0.05$) than the 5th instar of black soldier fly and the blow fly larvae. Similarly, lipase and proteinease activities of the 6th instar of black soldier fly were significantly higher ($P < 0.05$) than the 5th instar of black soldier fly and blow fly larvae. Blow fly larvae had a significantly higher ($P < 0.05$) values of amylase activity. Significantly higher ($P < 0.05$) concentrations of vitamins B, C, D and E were recorded in the 6th instar of the black soldier fly larvae. Na, K, P and Zn concentrations were significantly higher ($P < 0.05$) in the 6th instar larvae of the black soldier fly. Amino acids and fatty acids concentrations were not significantly different ($P > 0.05$) in the 5th and 6th instar of the black soldier fly larvae and blow fly larvae. The biochemical composition has showed that the 6th instar (pre-pupae) of the black soldier fly have the highest concentration of fats, proteins, minerals and vitamins.

Evaluation of the antidiabetic potential of a vegetable soup fortified with *Tenebrio molitor* protein hydrolysates

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Peptides obtained through enzymatic hydrolysis of insect proteins have been proved to exert antidiabetic bioactivity by inhibiting dipeptidyl peptidase IV (DPP-IV) and α -glucosidase, but their potential as bioactive ingredient is scarcely studied. In this work, a vegetable soup was fortified with antidiabetic peptides (proved to *in vitro* inhibit DPP-IV and α -glucosidase) obtained from *Tenebrio molitor* protein and was subjected to a thermal treatment (121 °C for 21 min). Dilutions at 0, 25, 50 and 100% of vegetable soup were considered. These sterilised samples were subsequently stored for 40 days to evaluate the influence of storage on the bioactivity these peptides have proved to exert. Furthermore, the effect on the bioactivity (*in vitro* inhibition of the enzymes) after *in vitro* gastrointestinal digestion both in water and in the vegetable soup over the protein hydrolysates and the native protein was evaluated (diluted 5% and adding pepsin and pancreatin). Results showed that peptides added into the vegetable soup did not lose their capacity to inhibit the enzymes after the thermal treatment. This bioactivity remained without modification after 40 days of storage. The DPP-IV inhibitory capacity of the peptides partially decreased after being submitted to digestion in water, but was maintained after digestion in the vegetable soup. The α -glucosidase inhibitory hydrolysate improved its bioactivity after the digestion, both in water and the soup. Higher inhibition was exerted by the hydrolysate after digestion compared to the native protein digested. These results prove that functional soups could be produced by fortifying basic products with protein hydrolysates coming from sustainable sources, increasing their commercial value and health-promoting properties. F. Rivero-Pino is carrying out a traineeship at EFSA from May 1st 2021 to April 30th 2022. The work described is not related to his current work at EFSA, it is published under the sole responsibility of the author and may not be considered as an EFSA scientific output.

Purine and uric acid content in edible insects

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Edible insects exhibit potential as a good food source for the ever-increasing human population; however, for approximately 1-4% of the western population suffering gout, insect consumption may be problematic. The study aimed to analyse the content of purines (adenine, guanine, xanthine, and hypoxanthine) and their metabolite (uric acid) in six insects (*Tenebrio molitor*, *Alphitobius diaperinus*, *Gryllus assimillis*, *Acheta domesticus*, *Locusta migratoria*, and *Blaberus discoidalis*) and meats (chicken, pork, beef, and salmon). Compared to meat, edible insects contained equivalent or higher amounts of total purine (3.23-13.22 g/kg edible insect dry matter vs 3.61-5.53 g/kg meat dry matter) and uricogenic purines adenine + hypoxanthine (1.55-8.86 g/kg edible insect dry matter vs 2.97-4.91 g/kg meat dry matter). The effect of insect species, culinary processing of insects, and sex on purine derivative and uric acid contents were also proved. Our data showed that edible insects contain substantial levels of purines that may be problematic for people suffering gout.

Comparison of fat oxidation during frozen or nitrogen atmosphere storage of black soldier fly larvae (*Hermetia illucens*)

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The production of insects as food and feed involves a logistic chain of storage and transport within or between companies in the insect sector. To retain the quality of intermediate or final insect products during storage and transport, appropriate stabilisation, e.g. cooling/freezing or drying, is required. While application of those technologies may retain the microbiological quality of the products for a short time, they are not always sufficient to preserve the chemical characteristics (e.g. fat oxidation) or they may cost too much. Hence, a tailored stabilisation method may be required for longer transport/storage. Therefore, the aim of this research (as part of the Horizon 2020 SUSINCHAIN project) was to investigate storage under nitrogen (N₂) atmosphere as an alternative storage strategy for dried black soldier fly (BSF) larvae in order to avoid fat oxidation. During a 4 day period, dried BSF larvae were stored in plastic bags under 100% N₂ atmosphere at 40 °C to mimic a long international transport under worst case conditions. Frozen storage in air (-18 °C) was included as comparison. Every day, primary and secondary oxidation were determined as peroxide value (PV) and p-anisidine value (pAV), respectively. Also, oxygen (O₂) concentration in the packages was monitored daily. Results showed that in both storage conditions, primary and secondary fat oxidation occurred. PVs remained below 2.0 meq O₂/kg fat for the whole storage period, but pAV increased gradually over time for both conditions. While secondary oxidation for frozen BSF larvae was slightly lower on all days, pAVs never rose above 10 in any condition, which is well below the maximum limit of 20, widely applied in the fat and oil industry. The reason for the (limited) fat oxidation is presence of O₂ in the packages (air still present in or entering through the package). To conclude, fat oxidation of dried BSF larvae cannot be avoided completely by packaging in N₂ or freezing at -18 °C, but both techniques allow a few days of storage/transport with limited fat oxidation.

Meta-analysis of the black soldier fly (*Hermetia illucens*) bacterial community using 16S rRNA gene amplicon sequencing

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Black soldier fly larvae (BSFL) belong to the most widely reared insect species as an alternative protein source. Bacteria in the larval gut can provide benefits for the animal, though some bacteria can also be pathogenic for the insect. Characterisation of the BSFL microbiota is important for the production of BSFL in terms of yield and microbiological safety. The study of bacterial communities has shifted from traditional culture-based methods towards next-generation sequencing approaches targeting a taxonomic marker such as the ribosomal RNA (rRNA) gene. Although these techniques have greatly improved our knowledge on BSFL microbiota and potential factors determining the microbial community composition, it remains challenging to identify general trends and draw general conclusions over the different studies performed. In this study, 16S ribosomal RNA gene sequence data sets from 11 studies were re-analysed using a uniform bio-informatic pipeline to gain better insights in the BSFL bacterial community, potential factors that influence its composition, and differences between the gut and the whole larvae microbiota. Core gut bacteria were found to be members of *Enterococcus*, *Klebsiella*, *Morganella*, *Providencia*, and *Scrofinimicrobium*. Further, the factors 'Study', 'Age' and 'Feed' significantly affected the bacterial gut composition. When compared to whole larvae, a significantly lower number of observed zero-radius operational taxonomic units and a lower diversity was found for gut samples, suggesting that the larvae harboured additional microbes on their cuticle or in the insect body. Universal choices in insect sample type, primer selection and bio-informatics can strengthen future meta-analyses and improve our understanding of the BSFL gut microbiota towards the optimisation of insect production.

Fate of *Staphylococcus aureus* inoculated during rearing of black soldier fly larvae (*Hermetia illucens*)

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Black soldier fly larvae (*Hermetia illucens*, BSFL) are promising insects that can efficiently convert organic waste streams into valuable components. These organic waste streams may contain foodborne pathogens, such as *Staphylococcus aureus*. In literature, *S. aureus* has been identified as one of the main bacterial pathogens associated with BSFL. To assess the fate of the food pathogen and its potential transmission to BSFL, inoculation trials were executed to monitor the dynamics of *S. aureus* during rearing of BSFL. To this end, the quantitative level of *S. aureus* was assessed over a 6-day course after inoculation of the food pathogen (3 or 7 log cfu/g) to the substrate (i.e. chicken feed) at the start of the experiment (day 0). The level of *S. aureus* found in chicken feed (in the absence of larvae) did not significantly change during the first two days of the experiment, but decreased after six days. The *S. aureus* counts of the substrate decreased faster when BSFL were present. Moreover, the level of *S. aureus* found in larvae was below the detection limit (2.0 log cfu/g) after six days, regardless of the contamination level. These results imply that BSFL exert a reducing effect on *S. aureus* counts of both substrate and larvae. To explore potential mechanisms resulting in *S. aureus* reduction, a collection of 178 dominant microorganisms isolated from BSFL were screened for their *in vitro* antimicrobial activity against *S. aureus*. Intriguingly, all six fungal isolates, all identified as *Trichosporon* spp., showed clear activity against *S. aureus*. These results suggest that the possible presence of *Trichosporon* spp. in the inoculation trials contributed to the noticed *S. aureus* suppression. Future research should examine the role of *Trichosporon* spp. in controlling (food) pathogens, such as *S. aureus*.

Proximate composition and analytical taste characterisation of eight edible insects

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Given the growing interest in edible insects, not only as alternative protein source but also for their potential technological advantages, it is important to understand their nutritional characteristics as well as their flavour potential to be able to develop products that can avoid consumer rejection. This study aims to describe and compare proximate composition and analytical taste of 8 edible insect species, being provided by a commercial supplier. They were, on one hand, *Tenebrio molitor* (TM), *Zophobas morio* (ZM), *Galleria mellonella* (GM) and *Alphitobius diaperinus* (ALD) in their larvae form, and on the other hand *Acheta domesticus* (ACD), *Locusta migratoria* (LM), *Gryllobius sigillatus* (GS), and *Blaptica dubia* (BD) in their adult stage. After 24 h of starvation, they were slaughtered by freezing at -20 °C. The total amino acid content and the total nitrogen content were analysed allowing the species-specific calculation of the nitrogen-to-protein conversion factor. In addition, the total lipid, moisture and ash content were analysed. In order to determine the taste characteristics, free amino acids were analysed to estimate the acid, bitter, sweet, umami and salty levels of each sample. All analyses were performed in triplicate and the effects were examined through one-way ANOVA. The specific conversion factors ranged from 4.1 (ACD) to 6.4 (ALD). It was observed that ALD and LM had the highest protein content (18.5 and 17.9% respectively) while GM had by far the highest fat content (22%) followed by ZM (13%). The highest mineral content was found in ZM (6.2%) whilst ALD was the highest in moisture (72.5%), closely followed by GS (71.2%). In terms of taste, clear differences were found, either mainly bitter insects such as ALD, GM and LM, or insects whose umami-flavoured amino acid content (LM, GS and GM) was about twice as high as the rest. The salty taste of BD, GM, ALD and ZM was found to be almost non-existent. The GM had the highest content of sweet-tasting amino acids and ACD and GS showed the highest content of amino acids with sour characteristics. Acknowledgements to The Research Foundation of Flanders for granting Cristina Pérez Santaescolástica with a postdoctoral scholarship (num.1222921N).

Proximate, techno-functional, physicochemical and antioxidant properties of three edible insect flours from South Africa

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The inaccessibility of food resources has been a challenge for many developing countries and thus difficulty providing adequate nutrition for their populations, and lack of protein may result in malnutrition. Given the expected rise in population by 2050 of 9 billion people, there is a need for elevated protein production and supplementation in the traditional food diet. Insects have been proposed as sufficient protein sources to help mitigate the resource shortages of increasingly demanding consumers. The present study aimed to assess the potential for edible insect flours of *Gonimbrasia belina*, *Hermetia illucens* and *Macrotermes subhyalinus* from South Africa as ingredients in food systems. The proximal, techno-functional and antioxidant properties were assessed. *M. subhyalinus* (52.74±1.47%) had higher amounts of protein compared to that of *G. belina* (46.70±0.82%) and *H. illucens* (34.90±0.47%). The fat content ranged from 6.36 to 27.93%, and no statistical differences were observed in the carbohydrate content of the edible insect flours ($P>0.05$). A statistical difference was observed in the colour difference of the edible insect flours ($P<0.05$). The water-binding capacity and oil binding capacity of *M. subhyalinus* flour was comparatively higher than the other two edible insect flour and ranged from 1.46 to 1.48 g/g, respectively. The antiradical activity against DPPH-RS was higher for *M. subhyalinus* (55.57%), followed by *G. belina* (37.44%) and *H. illucens* (3.63%). The Reducing power of the edible insect flours ranged from 0.26 to 0.6. This study showed that the edible insect flours thereof are suitable for developing new food formulations.

Production of a mealworm protein ingredients by using food processes applied in the pulse and legume industries

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Acceptability of edible insects by Western consumers is the main challenge for the insect industry despite their environmental, economic, and nutritional benefits. However, it was demonstrated that the integration of insects as an ingredient rather than whole represents an interesting opportunity to promote consumer acceptability. Generally, and on the same model as pulse and legume protein concentrates/isolates, edible insect protein ingredients are desired due to their nutritional and techno-functional properties. Nevertheless, the production of insect protein concentrate is challenging regarding its complex composition (lipids, proteins, chitin). Consequently, the aim of this work was to generate a mealworm (*Tenebrio molitor* (TM)) protein concentrate and isolate by adapting the conventional food processes used in pulse and legume industries. In this study, only results related to the production of TM protein concentrate (generate after alkaline extraction of protein) is presented. The production of TM protein isolate by isoelectric precipitation is currently under development. Blanching and TM protein extraction processes were optimised to minimise the impact on protein structures as well as their techno-functional properties. The process parameters used for soy and pea protein isolated were adapted for TM to obtain the highest protein extraction rates and purity, with an optimal solubility to facilitate the incorporation of this protein isolate in food formulations. Results showed that blanching process at 90 and 100 °C for 2.5 and 3.5 min were adequate to reduce the microbial contamination and to inactivate enzymatic browning. However, the blanching step induced protein aggregation and slightly reduced the purity of soluble proteins recovered in the concentrate (51.6 vs 59.6% for non-blanching TM). Furthermore, the solubility of the protein concentrates at pH 5, 7 and 9 was also impacted by blanching process with a reduction of around 30% compared to non-blanching TM, regardless of the pH. The next steps will consist of evaluating the impact of blanching process and isoelectric precipitation on the purity and solubility of the TM protein isolate.

Impact of Instant controlled pressure drop pretreatment on the texturing and drying of protein-rich yellow mealworms

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The exponential growth of the world's population, along with the shortage of our resources, require a rethinking of our dietary patterns, particularly our protein intake habits. In fact, insects are a good source of food and feed since they are high in protein and fat, have a high feed conversion ratio, emit few greenhouse gases, and follow a circular economic logic. Yet, social acceptance of whole insects as a food source remains a significant barrier; however, when processed into protein-rich food products or protein isolates, consumer perception may improve. This study is part of the RAFINSECT project, funded by the HDF region of France and aims to establish an insect biorefinery. The goal is to fractionate and valorise all biomass fractions such as proteins, lipids, and chitin by integrating them into a wide range of food and feed matrices, as well as cosmetic and packaging, while preserving the quality of fractions and being environmentally friendly and energy efficient. Thus, we are focusing on improving the transfer phenomena of various transformation phases, and this by the application of novel technologies such as instant controlled pressure drop (DIC), pulsed electric field (PEF), ultrasound, and supercritical CO₂. *Tenebrio Molitor* larvae were pretreated with DIC, a novel texturing method, at different pressure (1.5-4.5 bars)-time (15-35 s) profiles, then dried at 50 and 60 °C in a ventilated oven, with the aim of producing a protein-rich flour. The impact of texturing on drying kinetics, sorption isotherms, particle size distribution, specific surface area, and larval characteristics were explored. The obtained findings show that DIC-texturing considerably enhances the hot air drying of larvae. In fact, the shrinkage phenomenon, which is the principal cause of poor drying performance, was successfully overcome. Consequently, starting accessibility, effective diffusivity and drying time were significantly improved, thus resulting in lower process energy consumption and better dried product quality.

Dynamic vapor sorption isotherms and isosteric heats of sorption of two edible insects

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Cirina forda (Cf) and *Rhyncophorus ferrugineus* (Rf) are widely consumed insects in tropical Africa. Drying is one of the main conservation techniques to improve their availability for distant markets and outside their harvest seasons. In order to control their drying process, their storage conditions and to estimate their shelf-life, it is necessary to know the humidity that they develop at equilibrium in environments that may present various relative humidity. Sorption isotherms of these two insects were determined at 25, 30, 40 and 50 °C using a dynamic vapor sorption (DVS) device. Five isothermal sorption models were fitted to the experimental data, and Peleg equation was found to give the best fit for both insects. Both insects exhibited sorption isotherm of type III indicating monolayer-multilayer behaviour with limited hysteresis. According to the sorption isotherms to ensure a safe storage at 25 and 30 °C the Rf larvae must be dried at 8.8 and 8% respectively. Cf caterpillars must be dried to 9.5 and 9.4% for respective storage at 25 and 30 °C. Based on Kinshasa climate data when these two insects are left in contact with ambient air, their equilibrium moisture content can evolve to values higher than values guaranteeing a safe storage. This suggests that for better preservation the dried insects should be packed in airtight packages. Using Heiss-Eichner model, dry base moisture contents of 6.9 and 8.5% respectively for Rf and Cf were predicted to ensure the expected shelf-life of 12 months at 30 °C when insects are packaged in polyethylene films.

Suppression of methicillin-resistant *Staphylococcus aureus* by larvae of black soldier fly, *Hermetia illucens*

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Biological conversion of animal and plant wastes into ingredients of animal feeds using larvae of black soldier flies, *Hermetia illucens*, is a rapidly emerging environmentally sustainable technology. Unfortunately, contamination of harvested larvae with pathogenic microorganisms may present a serious problem for its wide-scale adoption. However, there is also mounting evidence that black soldier fly larvae have antimicrobial properties, including suppression of several common pathogens. Most existing studies focused on Gram-negative species, but there is also some evidence of negative effects on gram-positive species. Methicillin-resistant *Staphylococcus aureus*, or MRSA, are gram positive strains of *S. aureus* resistant to the antibiotic methicillin. Contamination of meat and dairy products by MRSA has resulted in foodborne infections of humans. We conducted laboratory experiments testing if black soldier fly larvae suppress MRSA on shredded potato substrate as a part of the study investigating their potential for valorising potato wastes. Potato substrate inoculated with MRSA, potato substrate inoculated with MRSA and containing black soldier fly larvae, and uninoculated potato substrate containing black soldier fly larvae were incubated for three and seven days. Samples were plated onto both trypticase soy agar and on Staphylococcus Medium 110 agar for enumeration of total bacteria and MRSA, respectively. Both total bacterial counts and MRSA counts were reduced in the presence of black soldier fly larvae, with reduction being much more pronounced for the latter. Our findings confirm antimicrobial properties of black soldier fly larvae, which is likely to improve biosafety of its harvested biomass. Furthermore, future identification of biologically active molecules responsible for this reduction may have important practical applications of its own.

Piloting edible caterpillars value chain development through improved drying and processing in Cameroon

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Edible caterpillars are widely consumed in Central and Eastern Cameroon. Consumers prefer to buy them fresh (alive). However, they are available only during 3 to 4 months per year, from June to September. The rest of the year, only smoked caterpillars are available on local markets, with lower nutritional value, less attractive to consumers and higher toxicity due to the presence of Polycyclic Aromatic Hydrocarbons resulting from traditional smoking. In order to add more value to edible caterpillars, there is a need to develop improved drying or smoking technics in the chain through the preparation of processed food targeting urban markets. To achieve this, Cameroonian NGO LIFT designed an improved stove able to dry up to 15 kg of fresh caterpillars. Tests with 2 species coming from Central Cameroon (*Imbrasia epimethea* and *Anaphea* sp.) showed that *Anaphea* dried for 4 hours can be stored at ambient temperature for 3 months without degradation. *Imbrasia* dried for 4 hours show signs of moisture after only two weeks, certainly due to the absence of smoke during the drying process. Further tests with modified stove allowing a limited exposition to smoke are needed in order to find the right balance between quality conservation and less toxicity for *Imbrasia*. Dried caterpillars flour processed with local mill showed a similar capacity of conservation. Organoleptic tests with consumers (60 adults and 20 children) showed a high appreciation and interest for donuts and porridge enriched with significant amount of caterpillar flour. These encouraging results show a high potential for value chain reinforcement through the integration of new actors and new products in Central Africa.

Cricket powder as ingredient in fresh-pasta: dough properties and pasta quality

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Crickets, in particular *Acheta domesticus*, have been shown to be a valuable and sustainable alternative protein source for humans due to high protein content, and the sustainability of their production. Although insects have been a part of human diets since millennia and remain a common food in many countries, insect consumption within Western consumers is limited due to consumers' disgust and neophobia. The use of insect powder – not directly recognisable – as ingredient in the food products might increase the insect acceptability and the propensity to consume them. Among cereal-based products, pasta popularity is increasing worldwide and different formulations have been developed to improve its nutritional profile, including the protein content. In this study, we evaluated the effect of the use of cricket powder (CP) in wheat-based fresh pasta. *A. domesticus* powder was produced at the DeFENS lab and blended to wheat flour with three ratios: 5:95 (5%); 10:90 (10%), and 20:80 (20%). Dough samples were characterised in terms of: mixing time and stability, as well as tenacity, strengths and extensibility. Fresh-pasta (length: 200 mm; width: 13.5 mm; thickness: 2.0 mm) was cooked (pasta:water ratio = 1:10) for 90 s and the water absorption, cooking losses (g of solid loss/100 g of pasta) and firmness were measured. Fresh pasta from 100% common wheat was used as control (CTRL). Dough development time and mixing stability decreased in CP doughs, regardless of the enrichment level. Moreover, CP led to an increase and decrease in tenacity and extensibility indices, respectively, indicating an increase in dough stiffness. The increase in protein and fibre contents may explain the increase such changes in dough properties. For fresh-pasta ('tagliatelle'), the maximum replacement level allowable was 10%, as higher inclusion of cricket flour led to excessive quality worsening. CP-enriched pasta had higher water absorption and cooking loss compared to CTRL. As regards pasta firmness, the 5% replacement level did not affect this index, instead it decreased at 10% replacement level. In conclusion, CP can be used in fresh-pasta without dramatically worsening the quality of the product.

Formulation and characterisation of biscuits enriched with termites (*Macrotermes subhyalinus*)

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Entomophagy appears as eating habits in Cameroon. However malnutrition, is known as one of the catastrophe found in developing countries. The main objective of this study is the valorisation of edible insects through the formulation of enriched biscuits. To achieve this, termites (*Macrotermes subhyalinus*) were collected in the surrounding markets, then some technological treatments were applied (sorting, winnowing, washing, drying, grinding). A portion of the treated termites was defatted to obtain de-oiled termite flour. Delipidated termite-enriched biscuits were formulated with Expert Design software. The chemical composition, the nutritional values and rheological parameters have been conducted on biscuits obtained. It emerges from these analyses that water, ash, lipid, protein, carbohydrate and energy contents of whole *M. subhyalinus* are respectively 6.96; 3.47; 43.12; 48.75; 4.66 and 601.44 g/100 g. Furthermore, the values of termite-enriched biscuits have been improved. Considering defatted termites and non defatted termite biscuits, we obtained respectively (1.14 up to 2.79 g/100 g) and (1.06 up to 3.05 g/100 g) concerning water content; concerning ash vales were (8.65 to 1.58 g/100 g) and (5.72-4.32 g/100 g). For crude protein content (1.18 up to 4.87 g/100 g) and (9.07 up to 4.50 g/100 g). For lipids we obtain (28.20 up to 17.18 g/100 g) and (23.08 up to 23.74 g/100 g) and finally with regard to gross energy the respective differences (460.06 up to 529.76 kcal/100 g) and (495.82 up to 501.38 kcal/100 g) were obtained. At the end of our work, it emerges that the enrichment of biscuits based on *M. subhyalinus* has made it possible to increase significantly the crude protein content and the energy value of the biscuits thus formulated. Those biscuits can be used for the reduction of undernutrition infant feeding.

Technological and nutritional suitability of local cereals enriched with termites (*Macrotermes subhyalinus*) for pasta

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This study, whose main objective is to contribute to the enhancement of termites and the ability of our local cereals to pastification through the formulation of fortified pasta was conducted. After collecting termites (*Macrotermes subhyalinus*) and buying cereals (corn, yagoua rice, sorghum) in the surrounding markets, these have undergone technological treatments (Sorting, Winnowing, Washing, Drying, Grinding). To achieve the formulation of termite-enriched pasta, a blending plan was developed using the Expert Design software what allowed us to obtain cereal formulas at different levels of incorporation of *M. subhyalinus*. Physical, chemical, nutritional and rheological analyses were conducted on the raw materials and the pasta formulated. These analyses show that the water, ash, lipid, protein, carbohydrate and energy levels of *M. subhyalinus* are respectively 6.96; 3.47; 43.12; 48.75; 4.66 and 601.44 kcal/100 g. Regarding the chemical composition of the different formulas, the pastas enriched with termites presented values ranged from (8.61 to 13.91 g/100 g MS) regarding water content. Then (1.69 to 2.57 g/100 g MS) for ash content; (7.68 to 20.72 g/100 g MS) for raw protein content; (3.48 to 16.63 g/100 g MS) for lipids; carbohydrate levels (60.34 to 87.82 g/100 g MS) and finally in terms of raw energy the respective differences (1,940.28 and 1,065.58 KJ/100 g) were obtained. At the end of our work, it emerges that our local cereals (rice, sorghum, corn) can be used for pastification. The fortification of pastas based *M. subhyalinus* has significantly increased the crude protein content and energy value of the pasta thus formulated, which can be used to reduce undernourishment.

Quality and functional properties of salt-fermented *Tenebrio molitor* and *Protaetia brevitarsis* larvae

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Providing a substitute for seafood seasonings, this study developed *Jeotgal* (traditional Korean salt-fermented seafood) through fermented *Tenebrio molitor* (FT) and *Protaetia brevitarsis* (FP) larvae, which are edible insects. FT and FP were fermented at 20 °C as whole and ground forms at 20, 30, and 40% salt concentration for 60 days. Quality properties (pH, salinity, aw, total bacteria count), antioxidant capacity, and an assay of muscle loss inhibition were examined. At a count of pH and total bacteria after 60 days of fermentation, the ground form of FT (5.77 and 3.4 log cfu/g) and FP (5.91 and 4.0 log cfu/g) had lower values than the whole form of FT (5.91 and 4.9 log cfu/g) and FP (6.99 and 4.9 log cfu/g). The addition of 20 to 40% salt showed 15 to 18% salinity in FT and 16 to 25% in FP. The pH tends to decrease as the salinity increases. The IC₅₀ values for DPPH radical scavenging activity were decreased from 7.71 to 5.44 mg/ml in FT and from 7.06 to 5.64 mg/ml in FP after salt-fermentation. At muscle loss inhibitory assay, FT fermented for 60 days showed a 20% reduction of luciferase activity (muscle degradation promoter), compared to a 9% reduction in FT which fermented for 1 day. Based on these results, edible insects can process *Jeotgal* to replace fish resources for sustainability. The insects that had been salt-fermented for 60 days showed enhanced antioxidant capacity and muscle loss inhibition.

Insect as feed/alternative protein sources & diet formulations

Insect farming in support of wildlife conservation efforts in the Okapi Wildlife Reserve, D.R. Congo

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The trade in bushmeat (i.e. wildlife harvested for human consumption) provides a critical source of income and protein to rural communities throughout the Congo Basin. The growing demand for bushmeat by a burgeoning human population has led to overhunting and threatens many wildlife species with extinction. For example, between 2011 and 2018, within the Okapi Wildlife Reserve (OWR), Ituri Province, Democratic Republic of the Congo (DRC), okapi, bush pigs, and several species of primate and forest antelope have experienced significant population declines due to illegal hunting. To protect the welfare of humans and wildlife alike, replacing bushmeat-based livelihoods with alternative income-generating activities that also provide an affordable source of protein to meet the population's nutritional needs is paramount. Because wild-harvested larvae of the African palm weevil (*Rhynchophorus phoenicis*) is a popular seasonal protein in the Congo Basin, palm weevil larvae (PWL) farming is one such alternative. Farms for Orphans and the Wildlife Conservation Society are collaborating on a pilot project evaluating the efficacy of PWL farming at the household (hh) level in three villages within the OWR. Community surveys indicated >93% of respondents consume PWL when in season, >80% of respondents were interested in PWL farming and >90% of respondents were willing to consume farmed PWL. A demonstration farm was built in the village of Epulu, 38 people were trained in PWL farming techniques and farming materials for hh farms were distributed. One bin of breeding palm weevils and substrate, costing \$3.20 USD, can produce 75 edible larvae (0.5 kg) every month. Thus, a small-scale PWL farmer harvesting 20 bins monthly can yield 10 kg PWL, bolstering hh nutrition. At \$0.10 USD/larvae, this same farmer can make a net profit of \$86 USD/month, compared to \$45 USD/month for a commercial hunter. PWL farming is a promising approach to provide alternative livelihoods and year-round access to a preferred protein to communities surrounding critical wildlife habitat in the DRC and beyond.

Lutein bioaccumulation and bioavailability in the black soldier fly larvae

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Lutein is a carotenoid involved in human eye health since it is concentrated in the retina and filters blue light from the sun's rays. This xanthophyll is also used as a natural colorant in the food industry, e.g. to colour egg yolk and chicken skin. In this study, we evaluated the possibility of recycling the lutein present in the co-products of fruits and vegetables thanks to the larvae of the black soldier fly (BSFL). Indeed, BSFL are known to be ideal candidates for waste disposal and animal feeding and we have shown that they can bioaccumulate high concentrations of bioaccessible provitamin A carotenoids. The purpose of this study was: (1) to assess whether BSFL bioaccumulate lutein when reared on vegetable substrates rich in this carotenoid; and (2) to compare the bioavailability of lutein in the substrates and the larvae, i.e. its ability to be absorbed by animals. BSFL were reared on either kale, parsley, endive or broccoli and lutein was measured by HPLC in both these vegetables and in the larvae. Lutein bioavailability was determined by *in-vitro* digestion. If reared on parsley or broccoli, BSFL accumulated two times more lutein than the substrates, seventy-two times for kale, and sixteen times for endive (concentrations expressed on a fresh weight basis). Lutein bioavailability was similar or lower in BSFL than in their corresponding substrates. However, lutein concentration in micelles obtained after *in vitro* digestion was similar or even higher (in the case of kale) in larvae than in substrates. In conclusion, BSFL can bioaccumulate high concentrations of bioavailable lutein when reared on substrates rich in this carotenoid. This makes lutein enriched BSFL a major asset to the animal feed industry, and therefore indirectly for human food. Indeed, by consuming products from farm animals that have themselves consumed these larvae, one could benefit from an additional supply of lutein.

Effect of feeding black soldier fly larvae products to slow growing broilers on performance and health

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Insects have gained increasing popularity as replacement for fishmeal, soybean meal or oil in poultry diets. However, rather than being a novelty, insects celebrate a comeback in poultry diets. Already the ancestors of our modern chickens, living in the forests, consumed them to meet their dietary needs. Unlike past times, today's industry offers a bandwidth of products such as live insects, insect meal and oil. In this context, black soldier fly larvae (BSFL) products have been suggested as promising feed ingredients. Moreover, components of BSFL such as lauric acid, antimicrobial peptides, and chitin are assumed to improve poultry health. However, their structure and abundance might be altered by processing steps and positive effects might be related to dietary inclusion levels. The present trial investigated the effect of BSFL products and inclusion levels in broiler diets on performance and health. At arrival, 1,728 one-day-old slow growing broilers (Hubbard JA757) were randomly assigned to 9 treatment groups with 8 replicates. A commercial broiler diet was used as the control group. In the second group, 5% of the dry matter feed intake (DMFI) was replaced by live BSFL. In the third group, 5% DMFI was replaced by a mix of 2/3 BSFL meal and 1/3 BSFL oil, to mimic the nutritional composition of live BSFL. The fourth and fifth group received the same quantity of BSFL meal or oil as group three, respectively. The same pattern of treatments was repeated in group six to nine with a replacement of 10% DMFI by live BSFL as a baseline. All dietary treatments were iso-caloric and formulated on digestible essential amino acids. Until d 49 the broiler performance and health was quantified by several parameters. The results support the adoption of utilising insects as feed from the forest to the feed trough. We will present the data of this trial evaluating the effects of BSFL products on broiler performance and health.

The influence of different diets on growth and nutritional composition of yellow mealworm

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The loss of food due to the globe's growing population becomes a pressing problem for the environment, economy, and scientists in the coming decades. Insects can be the tool/pathway through which agri-food by-products can become a high-quality, nutritious solution for small livestock and humans. The *Tenebrio molitor* larva is currently one of the insects permitted and reared on a larger scale in Europe. There are different by-products from the regional scale producers that could be used as feed for insects and provide more efficient nutrient bioconversion. This study aimed to evaluate the growth performance, feed conversion ratio (FCR), the conversion efficiency of ingested feed (ECI), nutritional quality, and the final composition of fats of yellow mealworm larvae grown on different by-products. The 5-week-old larvae were provided with different diets that contained by-products (wheat (WB) and rye bran, rapeseed meal (RM), and rapeseed, flax and *Silybum marianum* cakes). Larvae growth rate (weight and consumed feed) were registered weekly until the first pupation. Collected data were subjected to one-way ANOVA. The lowest/highest FCR based on a fresh and dry basis was 3.32 and 2.01, respectively. The ECI values were statistically different for larvae reared on different experimental diets (mean value 45.7%). The individual larva weight ranged from 112.26 to 132.60 mg (f.m.) for larvae fed on WB 70/RM 30 and WB 100 diet, respectively. For nutritional profile, protein and fats ranged from 43.6 to 53.4% d.m. and from 22.3 to 30.0% d.m., respectively. The protein conversion factor used in the present study was 5.41. The primary fatty acids in all 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). Our results provide new data regarding the use and efficient conversion of cheap by-products from the industry by yellow mealworms.

Chicken: feed or insects, eggs or meat?

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Feed production is the environmental hotspot in poultry chains. Alternative feed sources of protein are required to reduce the ecological footprint. This research aims to identify environmentally beneficial way to produce chicken protein. Two systems of chicken protein production are compared: one directed towards egg and one towards broiler production. In comparison we considered 3 different scenarios for each of the system (reference scenario with typical chicken rearing system, based on commercial feed; 2 alternative scenarios with a part of feed protein substituted by black soldier fly larvae (BSFL)). Insect production in a turn relied on two diets: Gainesville diet and fruit and vegetable waste (FVW). The study followed the cradle to slaughterhouse gate (or egg production gate) perspective including feed production, hatchery, poultry and egg production and slaughterhouse. Two functional units were: protein conversion ratio, and assessment of the production of 1 kg of poultry protein. Data used in this study are based on literature; life cycle assessment (LCA) was developed using a modular and attributional approach. Results of LCA showed higher environmental impacts in laying hen production (11.8 mPt per kg protein) than in broiler chicken production (9.95 mPt per kg protein). This was predominantly due to the composition of feed; additionally, the environmental impact per bird is higher in layer production than in broiler chicken production due to the longer life cycle. Scenarios that supplemented BSFL in the feed improved production in both cases, with FVW fed BSFL performing slightly better, as no environmental impact was attributed to the production of the waste.

Guinea Bissau and Kenya: two examples of cooperation to implement the use of insects as feed

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The protein shortage for feed is a global issue, but it affects in particular the rural areas of developing countries where access to raw materials is less and less sustainable. The volatility of commodity prices and their continuous rise has a huge impact on small businesses. Feed is the main constraint for African farms, and the current challenge is to contain it to ensure their economic sustainability. The insects, and in particular the black soldier fly larvae (BSF), are easily bred within the climatic conditions of different regions of Guinea Bissau and Kenya. This allows the production of low-cost, high-nutritional proteins for poultry, pigs, and fish feeds, thus ensuring the growth of small- and medium-sized farms in rural areas. At the same time, it allows the re-use of production waste that would be lost (circular economy). Two projects have been developed with the collaboration of the University of Turin, Mani Tese (Italian NGO), and local NGOs. In Guinea Bissau, two small BSF pilot plants have been created at social enterprises in Bissau (Cedaves) and Canchungo (Piu Piu Awara), aims at producing live larvae for poultry production. The project is implemented in partnership with IMVF (Instituto Marquês de Valle Flôr) and the local NGO 'Asas de Socoro' (project Ianda Guiné! Galinhas, funded by the European Union). In Kenya, the collaboration (project AGRICHANGE, funded by the Italian Agency for Development Cooperation) aims at the implementation of an already existing BSF small farm to support the pig sector in Molo (Nakuru county), and Marigat (Baringo county). In Kenya, the collaboration is carried out with the local NGO Network for Ecofarming in Africa (NECOFA). The support of these entrepreneurial realities could result not only in very practical economic implications (decrease in the cost of feeds), but could also and above all have a huge social impact (access to training for women, youth entrepreneurship, guarantee of income).

Piloting insect-to-fish farming systems in Nigeria

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Within small-scale aquaculture value chains, a key challenge entails securing access to affordable and environmentally sustainable protein sources for fish feed. This need is critical, especially in Nigeria, where current fish production is insufficient to meet market demand. Small-scale farmers are 94% of the Nigerian fish farming sector, for whom the main hindrance in fish production is the scarcity of and high costs associated with fish feed. Fishmeal comprises ca. 50% of aquaculture fish diets and represents 40-70% of total operational cost. Fish farmers often replace animal protein in fishmeal with less expensive grains, but the current supply of good quality seed is inadequate, unsustainable, and costly for the everyday small-scale fish farmer. There is an alternative source for quality, reliable, sustainable, and safe protein to be used in fishmeal preparation – insects. We are developing an integrated insect-to-fish (ITF) farming system and evaluating the economic, social, and biological potential for scaling ITF to improve the affordability, accessibility, and quality of feed, and therefore livelihoods and food security in Nigeria. Our approach is developing insect farms to work in synchrony with local fish farmers to teach the community how to mass rear insects, specifically, the black soldier fly (BSF), *Hermetia illucens* (L.) (Diptera: Stratiomyidae). Ultimately, project outcomes will reduce reliance on crop production and increase availability of fish for consumption, while stimulating small business enterprises driven by women and youth. Integration of these two industries will contribute to solving the need for alternative fish feed protein replacement for aquaculture systems.

Local and regional black soldier fly rearing potential in Uganda

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Black soldier fly larvae (BSFL) rearing is becoming an alternative source of protein for livestock feed globally, providing cost savings in poultry, aquaculture and swine production. In developing countries with high competition for declining fish meal, BSFL rearing using organic waste is perceived as an environmentally friendly initiative for adoption of economically challenged youth and women. However, there are important considerations and a need for improved understanding of BSFL rearing in local communities of Uganda. Using a mixed research method, an experimental BSFL unit was established by the Center for Insect Research & Development with capacity to generate one tonne BSFL feed per week. Six youths were employed to run the unit under a business model to assess profitability and autonomy. After five months of operation, the unit generated revenue to cover operational costs, and created demand for BSFL products like breeding stocks of eggs and pupae and dried larval feed for commercial livestock production. Over 7 months, more than 100 early starter BSF farmers were trained. Our initial effort attracted interest and visits by ministers and leaders of national government programs promoting BSFL for sustainable agro-industrialisation of poultry and swine value chains in Uganda. Challenges have been securing reliable, sorted sources of organic waste to feed the larvae; unsorted waste poses hazards to employees (e.g. razor blades, needles). New strategies of organic matter acquisition, quality and improved safety will be needed to accelerate BSFL rearing within rural settings of Africa. Thus far, operations are managed by manual labour, but there is need to investigate affordable approaches of automation (e.g. harvesters, dryers). In summary, BSFL rearing has potential to be a profitable venture in Africa, and should be promoted as an alternative value chain among peri urban youths and women entrepreneurs.

Near infrared spectroscopy for the prediction of nutritional composition of black soldier fly larvae

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The demand for sustainable animal feeds is increasing due to prevailing socio-economic conditions and several other underlying factors. Black soldier fly larvae (BSFL) are considered to be a viable option owing to their ability to forage different organic matter. BSFL reared on organic waste streams are rich in nutrients including various essential amino acids and fatty acids required for animal feed. BSFL nutritional composition is impacted by feed, rearing conditions and other processing factors throughout the production line. A rapid non-invasive technique to determine the nutritional composition of BSFL would be valuable to monitor the quality of the larvae reared in large volumes for commercial practices. The use of Near Infrared (NIR) spectroscopy as an analytical tool for determining the nutritional composition of food and feeds is well explored. Thus, the objective of this study was to determine the ability of NIR to predict the proximate composition (crude protein, chitin, lipids, and fibres), plus amino acid and fatty acid profile of BSFL and pre-pupae reared on soy waste. BSFL (n=50) and pre-pupae samples (n=50) obtained from a commercial BSFL production facility were analysed by NIR. Additionally, proximate analysis, amino acids and fatty acid analyses were conducted on these samples by standard analytical methods. The NIR scans obtained from these samples were developed into calibration models using chemometric techniques such as principal component analysis and partial least squares. The outcomes of this study will help to reduce the cost incurred for conventional analysis, while also providing guidance in selecting the suitable time frame for larvae harvesting based on their nutritional profile.

Slaughtering performance of medium-growing chicken fed black soldier fly live larvae

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This study aims to evaluate the effects on slaughter performance of medium-growing chickens fed live insect larvae. A total of 240 Label Naked Neck chickens were reared from 21 to 82 d of age. The birds were split according to gender and dietary treatment in 4 experimental groups (6 replicate/treatment, 10 birds/pen). The treated groups received 10% supplementation (of the expected daily feed intake) of black soldier fly larvae. At 82 d of age, 2 birds/pen (12 birds/treatment) were selected on the basis of the pen average body weight (BW) and slaughtered. The ready-to-cook carcass weight (RTCCW) was recorded. The weight of the heart, spleen, bursa of Fabricius (BF), liver, gut, and stomachs were collected (%BW). After 24h of refrigeration (4 °C) the chilled carcass weight (CCW) was recorded. The carcass yields (%BW), breast yields (%CCW), thigh yields (%CCW), water losses (WL) (%RTCC-CC, 24 h after slaughter) were calculated. The pH and colour (after 24 h at 4 °C) of breast and thigh meat were registered. Data were analysed using a GLMM ($P < 0.05$). The spleen weight was higher in both treated groups than controls ($P < 0.01$), attributable to larvae immune activity. Treated females showed a higher BF weight than control females ($P < 0.05$). Moreover, treated birds revealed a higher absolute heart weight than control ones ($P < 0.05$). Finally, WL were lower in females fed larvae than control ($P < 0.01$). In conclusion, live larvae may be safely used in medium-growing chickens feeding programs and may exert the stimulation of immune system.

Digestibility of defatted insect meals in rainbow trout aquafeeds

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The assessment of the apparent digestibility coefficients (ADCs) of defatted insect meals (IMs) is fundamental for the correct inclusion of these innovative protein sources in diets for carnivorous fish species. This study aimed at assessing the ADCs of main nutrients, amino acids and fatty acids (FA) of four defatted IMs (two from yellow mealworms, one from black soldier fly and one from lesser mealworm) in rainbow trout (*Oncorhynchus mykiss*). Five experimental diets were prepared, a control diet and four diets containing 30% of each tested IM based on the substitution method. Celite® was used as digestibility marker. The trial was performed on 80 rainbow trout (initial body weight: 140±5.6 g) kept into tanks connected to in flow-through open water system. After 10 days of diet adaptation, fish faeces were collected across four consecutive weeks using an automatic Choubert device and then analysed. Significant differences were observed among the IMs for the ADCs of dry matter, crude protein, gross energy, methionine, cysteine, glycine and tyrosine, with the lesser mealworm meal showing the overall lowest digestibility values. Such differences were imputed to both insect species and meal processing techniques. The ADCs of ether extract (in all cases higher than 96%) and main FA (namely lauric, myristic, palmitic, oleic, linoleic and α -linolenic acids; in all cases higher than 85%) did not significantly vary among the IMs. Based on the results, the tested IMs resulted highly digestible and could be a valuable protein source for the formulation of sustainable rainbow trout feeds. Funded by SUSINCHAIN H2020 Project.

Improvements in growth, welfare and gut microbiome in broilers supplemented with BSF larvae at commercial scale

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The global chicken meat (broiler) industry has been intensively criticised by the media in terms of animal welfare, as well as of sustainability due its dependency on soybean from South America. As chicken meat remains one the major source of protein in peoples' diet, it is important to make its supply sustainable while maintaining an accessible price. In this scenario, black soldier fly larvae (BSFL), as a feed component, have the potential to deliver a solution for the broiler industry. BSFL can be raised locally on food waste, it is an excellent source of protein and has been used as a welfare enrichment for a variety of livestock systems. This study aimed to confirm that BSFL can be used to supplement broiler's diets at a commercial level, as well as investigate their benefit in terms of growth, welfare and gut microbiome composition. Thus, 15,000 Ross 308 broiler hatching eggs were equally divided into two sheds, where upon hatching one was fed with chicken feed only (control) and the other supplemented with BSFL at 5% (w/w) for 14 days. On the 7th and 14th days, welfare evaluations were conducted and 10 chickens from each shed were weighed and euthanised for sampling collection. Microbiome analyses were performed on samples of caecal contents focusing on the 16S V3-V4 region. After 7 d, BSFL-fed broilers were 16% heavier than the control. Factory processing chicken weights were on average 4.63% higher in the BSFL-fed shed (6 weeks), even after the supplementation stopped. During the welfare assessments, broilers in the BSFL-fed shed were more approachable, less flighty, and spent more time foraging than in the control. Moreover, the gut microbiome of BSFL-fed broilers was more diverse than the control at 7 d, but diversity significantly decreased at 14 days in both groups. In particular *E. coli* and *Shigella* were more abundant in the gut of control broilers than in the BSFL-fed, which is of great importance for reducing the use of antibiotics. These results emphasise that early supplementation of broilers with BSFL improve their welfare, growth and microbiome composition.

Partially defatted black soldier fly meal inclusion in Pacific white shrimp diets: effects on growth 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. Insects make it possible to valorise food waste (bioconversion). The black soldier fly (*Hermetia illucens*) represents a particularly adapted resource to feed fish or shrimps because it is natural, safe, sustainably produced and has a good nutritional quality. The objective of two studies carried out in the facilities of IMAQUA (Belgium) in 2020 and 2021 was to evaluate the zootechnical performances of shrimps (*Penaeus vannamei*) when a part of fishmeal is replaced by a partially defatted black soldier fly (BSF) meal in comparison to a conventional feed (CTRL). In a first trial, a BSF meal, produced by a French company (MUTATEC), is incorporated in pelleted feeds at different inclusion rates (6.4; 12.7 and 19.1%) as replacement material for fishmeal (respectively 33; 66 and 100% of replacement). These feeds have been used to feed Pacific white shrimps from 0.24 grams to 2.74 grams (28 days). In a second trial BSF meals from two processes were incorporated in the feeds to replace 66% of the fishmeal. These feeds have been used to feed shrimp from 0.93 grams to 9.86 grams (48 days). Although not significant, an increased final weight (and related weight gain) was observed in all treatments where fishmeal was replaced with insect meal. In the first trial, the optimal inclusion level was 12.7% (66% of fishmeal replacement), the specific growth rate was significantly better than the CTRL for this group of shrimps. An increase in average final weight by 16.8% for this group could be observed after 28 days of feeding. At the end of a complete culture period this would result in a substantial increase in productivity. These results suggest a positive impact of BSF meal on growth performance in shrimp. It therefore seems possible to replace a significant portion of fishmeal by BSF meal. These trials ended with a bacterial challenge which showed promising results that will be presented later.

Influences of fish and defatted black soldier larvae meal diets on the growth of juvenile Chinese soft-shelled turtles

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The black soldier fly larvae meal (BSFM) has been widely applied in aquaculture production due to its rich nutritional value, easy availability and renewability. However, to our knowledge, it has not been used in the diets for turtles. Here, we evaluated the acceptability of partial substitution of fish meal (FM) with defatted BSFM in the diets of juvenile Chinese soft-shelled turtles (*Pelodiscus sinensis*). A total of 225 juvenile turtles were randomly divided into D1, D2, D3, D4 and D5 groups, which were fed with BSFM replacing 0, 5, 10, 15 and 20% FM, respectively, for 70 days. The results showed that growth performance of groups D4 and D5 were significantly different ($P<0.05$) from that of group D1. The activity of serum alkaline phosphatase was higher ($P<0.05$) in group D3 relative to other groups, while alanine aminotransferase and aspartate aminotransferase activities were higher ($P<0.05$) in group D4 when compared with groups D1, D2 and D3. Muscle crude protein and fat contents increased ($P<0.05$) in group D3 when compared with groups D1 and D2. Muscle phenylalanine, glutamic, tryptophan and arginine levels increased in group D1 in comparison with the other groups ($P<0.05$), whereas the opposite was true for isoleucine and proline levels. The broken-line analysis based on specific growth rate estimated that the optimal level for replacing dietary FM with BSFM is 5.0%, which could elicit benefits on both the growth performance and physiological health condition of the juvenile Chinese soft-shelled turtles.

Insect meal in layer chicken diet enhances growth, egg production, egg quality, meat quality and farmer profitability

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Due to the scarcity and high cost of major protein sources in poultry diet, black soldier fly (BSF) larvae (*Hermetia illucens*) meal has been widely accepted globally as suitable alternative. This study evaluates the effect of substituting soyabean meal and sunflower cake with BSF meal on growth, feed intake, feed conversion ratio efficiency, egg production, egg quality, spent hen meat quality and profitability. Our results revealed that chicks and growers fed diets containing 75 and 50% BSF meal, respectively, had the highest overall live body weight gain. Chicks and growers fed diets with 100% fishmeal recorded the highest average feed intake. Although, layers fed diet with 75%BSF meal inclusion recorded the lowest feed intake, they presented the highest live body weight. Layers fed diet with BSF meal recorded 2-4% increase in egg production compared to those fed diet with 100% fishmeal. Egg production and egg size showed a quartic and cubic response, respectively, with maximum estimate at 89.9 and 73.1% inclusion levels of BSF meal. Almost all the physical properties of eggs from the layers were observed to increase with increasing inclusion of BSF meal. The crude protein content of eggs from layer fed diets with BSF meal ranged between 38-40% compared to 32% for those fed diet with 100% fishmeal. No significant effect of diet types was observed on the proximate composition of spent hen meat powder. α -linolenic (omega-3) and linoleic (omega-6) acids were the most dominant polyunsaturated fatty acids. Eggs collected from birds fed diet with 100% BSF meal had significantly higher iron and zinc contents. The most cost-effective diet was that with 75% BSF meal. This implies insect-based feed could be a novel solution to adequately overcome the protein gap in poultry feed industry.

Effects of *Desmodium intortum* and black soldier fly larvae-based meal on sensory and physicochemical parameters

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We evaluated the effect of dietary inclusion of different ratios of *Desmodium intortum* (DI) and black soldier fly larvae meal (BSFLM) on sensory and physicochemical qualities of broiler chicken meat. Three formulations: T1 = 75%BSFL:25%DI; T2 = 50%BSFL:50%DI; and T3 = 25%BSFL:25%DI as a protein source were tested. A conventional feed was used as a control diet. On day 42, the chickens were slaughtered, and thigh muscles were tested for sensory and physicochemical evaluations. T1 was preferred for its flavour and tenderness, T2 for its juiciest and T3 for its colour, flavour, taste and overall acceptability scores. In terms of physical properties, all three types of meat had significantly lower moisture content and a higher water holding capacity than the control. T3 had the lowest moisture content, cooking loss and pH whereas the meat from control diets had the highest cooking loss and pH. No significant differences were found in terms of meat lightness; however, meat from control diet showed the highest redness value, and T3 had the highest yellowness value. Dietary inclusion of DI-BSFL affected the fatty acid and cholesterol profiles of the meats. A holistic principle component analysis indicated that the tested meats were distinct in their sensory and physicochemical properties. These variations in physicochemical properties may account for the difference in organoleptic perception, especially for T3. Further research should focus on feed modulation in relation to consumers preference and potential health benefits.

Updated insights on marketing of insect as feed focused on insect-based pet food applications

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The increase in pet ownership has driven the rising demand for pet food production which currently encumbers up to one fifth of global meat consumption – directly competes with human food production and contributes to significant amounts of greenhouse gas emissions. Commercial pet food production emphasises quantity over quality, creating doubts about the source and quality of ingredients as well as additives and their health effect on pets, including the rise of diseases, digestive disorders and allergies. Insect-based pet foods have been proposed in recent years as a nutritionally complete alternative that is palatable to pets and has a potentially significantly lower environmental footprint than conventional nutrient sources. Insect-based pet foods as a segment has been growing fast in the last 2-3 years, primarily in the form of dry pet food, and recently received endorsements from industry associations such as BVA and AAFCO. However, the segment is still in its infancy and questions remain about the potential of insect-based pet foods to become mainstream, including questions around their palatability, digestibility and health effects. In this study, we present an overview of the related literature and present the results of multiple controlled and semi-controlled trials conducted by our group to assess palatability, digestibility and health & behavioural effects of insect-based wet dog food formulations (black soldier fly, *Hermetia illucens*) based on AAFCO nutritional guidelines.

Nutrient digestibility of diets containing five different insect meals in gilthead sea bream and European sea bass

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Given the growing interest in the use of insect meals in aquafeeds, it is advisable to evaluate their nutritional value for the farmed species by assessment of digestibility and efficient utilisation ability. The study aims to evaluate and compare the nutrient, amino acid and energy apparent digestibility coefficients (ADC) of diets which included 19.5% insect larvae meals from *Tenebrio molitor* (TM), *Hermetia illucens* (HI), *Musca domestica* (MD), *Zophobas morio* (ZM) or *Alphitobius diaperinus* (AD) in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). A diet in which fish meal (65%) was the only protein source was used as control (FM). Similar or slightly better digestibility was observed in diets TM and MD for European sea bass due to higher adjusted protein ADC, while the other nutrient ADCs were similar to the FM diet. Diets HI and AD exhibited worse overall digestibility compared to FM, with similar or lower ADCs. The ZM diet displayed moderate results with lower dry matter ADC compared to FM but higher protein ADC. Nonetheless, the differences in ADCs between the six diets were minute. Regarding gilthead sea bream, no differences were observed in the nutrient and energy ADCs, except for TM which had the lowest fat ADC. All amino acids were highly digestible in both fish species. Additionally, the digestibility of the sum of amino acids was not affected by the inclusion of different insect meals. In conclusion, our results show that, regarding nutrient digestibility, diets containing 19.5% of these insect meals are suitable for both fish species. This study was funded by European Union and Greek national funds through the National Strategic Reference Framework 2014-2020, Special Actions Aquaculture-Industrial Materials-Open innovation in culture (code: MIS 5045857, acronym: Entomo4fish).

Transfer of aflatoxins and heavy metals to egg and meat of laying hens fed fly larvae reared on contaminated substrate

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The use of low-grade substrates can improve the sustainability of insect-based feed ingredients but also poses food safety risks. These include mycotoxins and heavy metals present in substrates and insect-based feed, thereby being transferred to recipient animals and further into the food chain. We studied the transfer of selected contaminants to black soldier fly larvae (BSFL) and poultry-derived food. Four poultry diets were formulated from 4 partially defatted meals produced at 2 different facilities. In Indonesia, BSFL were reared on food waste containing animal by-products spiked with environmentally relevant concentrations of Cd (1.9 mg/kg), Pb (19 mg/kg) and aflatoxin B1 (1.5 mg/kg), next to a non-contaminated control. As an additional control, in Switzerland, BSFL were reared on substrates approved in the EU. Defatted BSFL were included at 200 g/kg diet for late-laying hens (n=9/treatment), and fed for 4 weeks. Only the diet including BSFL reared on Cd contaminated substrate exceeded the EU maximum level for Cd for complete feed (1.7 mg/kg vs 0.5 mg/kg). No diet affected laying performance or egg quality. Feeding heavy metal contaminated BSFL-based diet doubled Cd concentrations in breast meat and elevated Cd concentrations in kidneys and liver compared to the control. However, all respective poultry products and tissues (except kidneys) ranged below permitted limits for food. Our results show that, under certain conditions, even contaminated food can provide a suitable substrate to produce BSFL for use as feeds for poultry nutrition.

Modulation of medullary body morphology and positive remodelling of artery by BSF fed chicken femur bone

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Black soldier fly (BSF) larvae and pre-pupae can be raised on the household organic waste and used as poultry feed. Previous research indicated that BSF oil did not affect the body weight and egg-laying rate, but the eggshell thickness was significantly higher in the pre-pupae fed group. Replacement of soybean meal with BSF larvae and pre-pupae affects eggshell strength, and induces specific morphological changes in bone tissue. To prove the previous hypothesis, we analysed bone histology and structure using micro and macro trace by x-ray analytical microscopy and X-ray micro-computed tomography (μ CT) methods on medullary formation in the chicken femur bone. The histological results show that BSF-supplemented feed accumulates TRAP-positive cells in chicken medullar bodies. The micro-CT images detected the connecting shape epiphyseal arteries on the femoral head to neck in both BSF-fed groups. The X-ray analytical data indicated that Ca, Fe, and Zn were slightly increasing and colocalised in the femur bone head. SEM observation has shown enlargement or positive remodelling of bone blood formation in BSF-fed chicken, which might promote the calcification in the vessels. Therefore, we speculated that the feed-derived BSF preparation-promoting ingredient might have increased the absorption of dietary minerals. Later studies of μ CT morphology indicate that blood flow properties influence medullar bone remodelling in the chicken femur.

Effect of rearing substrate on growth performance and body composition of black soldier fly larvae

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In the last years, the black soldier fly (BSF) has received increasing attention due to their larvae's capacity to convert a wide range of organic wastes into biomass that can be used for feed. However, the type of waste can have major effects on the total larval harvest yield and their nutritional quality, which are important determinants for efficient insect production. Previous studies showed that larvae reared on chicken feed or Gainesville housefly feed have the fastest larval growth, however, these substrates also compete with current food and feed systems. The focus of the study was to identify potential organic waste and by-product streams locally available in Denmark that could support larval growth and possibly modify the fatty acid profile to make them more a more valuable feed ingredient. In this study, 5-day old BSF larvae were reared at an industrial scale for seven days on different organic wastes and by-products locally available in Denmark: brewer's spent grain, mitigation mussels (*Mytilus edulis*) – used previously for water filtering, shrimp waste meal (*Pandalus borealis*), and rapeseed cake. Larval growth and composition were compared to chicken feed and a reduced chicken feed diet (48% pea grits, 24% wheat, 21% chicken feed, 6% sugar beet pellet, 1% vitamin-mineral mixture); the latter containing a similar macronutrient composition to chicken feed but with partial replacement using by-products. Larvae fed either brewer's spent grain, mitigation mussels or shrimp waste had a lower final biomass yield when compared to larvae fed the other substrates. It is hypothesised that the impaired larval growth was due to the lack of digestible carbohydrates in these diets, being the main energy source in nature for this species. However, these larvae had a significantly higher protein percentage and lower total lipid percentage. Larvae fed the mitigation mussels and shrimp waste had a significantly higher omega 3 fatty acid content. However, most fatty acids were saturated in larvae of all substrates indicating that the fatty acid composition can be only modified to a certain extent, suggesting that the lipid fraction may not be suitable to completely replace current lipid sources in feed such as fish oil.

Fatty acid profile, physical and sensory characteristics of eggs produced by hen fed on black soldier fly larval meal

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Provision of poultry feeds comprising of all the dietary requirements is a major challenge to the poultry industries. Insects have been used as an alternative source of protein for poultry production. This research aimed to provide evidence on sensory characteristics and nutrient quality of eggs produced by laying hen fed with defatted black soldier fly larvae meal (BSFLM). Feed formulation with BSFLM and Fish meal (FM) was as follows: Diet₀ (100% FM + 0% BSFL meal; Control diet), Diet₂₅ (75% FM + 25% BSFL meal), Diet₅₀ (50% FM + 50% BSFL meal), Diet₇₅ (25% FM + 75% BSFL meal) and Diet₁₀₀ (100% BSFL meal + 0% FM). The source of protein and fat for the control diet was the FM. This study evaluated Fatty acid profile, physical properties, and sensory characteristics of eggs produced by hens fed on a diet supplemented with BSFLM. Isoenergetic and Isonitrogenous insect-based diets were introduced to two hundred and fifty birds in which five (5) birds were assigned per replicate for each treatment during the egg laying period for eighty weeks. Egg weight ($P < 0.001$) and yolk weight ($P < 0.001$) significantly increased with Increased levels of BSFLM in the diets. The dietary linoleic/ α -linolenic ratio (n6/n3) ($P = 0.119$) and polyunsaturated fatty acids ($P = 0.073$) were not affected by the inclusion of BSFLM in the diet, while the content of monounsaturated fatty acids ($P < 0.001$) significantly increased with the increase of BSFLM levels in the diet. Boiled eggs were subjected to the sensory assessment within 24 hours after collection. Five samples were coded with 3- digits, and one sample was indicated as the control and presented to 50 trained panellists. Sensory attributes evaluated were flavour, aroma, and off-flavour and yolk colour using a 9-point intensity scale. The egg yolk colour ($P < 0.001$) improved significantly with the increase of BSFLM in the diet while flavour, off-flavour and aroma were not significantly affected. Our research findings implied that incorporation of BSFLM into the layers diet could replace FM while maintaining sensory characteristics and egg quality.

Results of palatability, digestibility and maintenance with black soldier fly larvae ingredients in dog food

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North American production of black soldier fly larvae continues to grow, providing an alternate sustainable and hypoallergenic source of protein and fat for pet food industry. As consumers increased awareness of and demand for more sustainably produced ingredients including insects, pet food manufacturers need to understand how incorporating black soldier grub ingredients into their portfolio can help create a competitive advantage. In this presentation Enterra will provide an overview of the most recent technical data including acceptance, palatability, digestibility and maintenance trial results from the past year. Highlights include: Acceptance: Over 10 days, 8 dogs consumed 100% of the black soldier fly larvae based dog food presented to them. Palatability: Over 10 days, there was no significant difference in amounts eaten of the black soldier fly larvae based dog food (110 g intake per day) vs the standard fish based dog food (125 g intake per day). The black soldier fly larvae food was the preferred dog food in 5 out of the 10 days. After adjustment for moisture content which can have a significant effect on the palatability of dog food, the results show a resounding preference for the black soldier fly based diet. Over 4 days, there was a significant difference in amounts eaten. The black soldier fly larvae based dog food (193 g intake per day) was clearly preferred by the 10 dogs in this study vs the red meat based dog food (77 g intake per day). After adjustment for moisture content, which can have a significant effect on the palatability of dog food, the results are even more resounding for the black soldier fly based dog kibble. Digestibility: Over 8 days, digestibility results of black soldier fly/red meat based diet showed excellent digestibility, with an average of over 80% digestibility for dry matter and protein digestibility, and over 90% fat digestibility. Also, faecal scores were excellent, with all stools being well formed with visible grooves. Maintenance: Over 26 weeks, dogs were fed a black soldier fly larvae based diet. All health metrics were excellent throughout the study, including maintenance of body weights, body condition scores and strong results of bloodwork showing no concerns at all.

Black soldier fly product-specific interactions with *Lactobacillus plantarum* in the diet of layers hens

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Previous study compared the effects of three diets (corn-soybean diet (Control), 7% black soldier fly defatted meal (BSFd) diet and 7% black soldier fly whole larvae meal (BSFw) diet) on layers production parameters, egg morphology and health status. Layers responded specifically to the BSF-based diets with several positive effects on the production parameters. However, both BSF-fed groups produced smaller eggs compared to the control (corn-soybean diet), with significant differences recorded in the group fed with the BSFd-based diet. The aim of this study was to test if the inclusion of *Lactobacillus plantarum* in the BSF-based diets would have a positive impact on hens' production parameters and health. A total of 240 layers hens (60 hens/group; 20 hens/pen) were assigned to four dietary treatments – Control – 7% BSFd meal (BSFd), Treatment 1: 7% BSFd meal with 0.1% *L. plantarum* (BSFd+pro), Control 2: 7% BSFw meal (BSFw), Treatment 2: 7% BSFw meal with 0.1% *L. plantarum* (BSFw+pro) to test the above hypothesis. Hens were fed for five weeks (age 50–55 weeks). Results suggest that *L. plantarum* interacts differently with the two BSF products, resulting in different outcomes. For instance, BSFd+pro-fed hens produced larger eggs (week 5; -58.79) compared to the control 1 group (week 5: 56.32 g; $P=0.007$), while hens fed with BSFw+pro diet produced eggs with similar weight to the control 2 group (wk5 57.86 g vs control 2 57.43 g; $P=0.613$). An array of blood serum parameters were analysed as well, indicating no negative impact on layers' health status. The study demonstrated two important points: firstly, *L. plantarum* interacted differently with the BSFd- and BSFw-based diets to produce specific responses in layers; secondly, the combination of a probiotic with BSF products may improve layers' performance.

Use of yellow mealworm as a protein source on growth performance in growing lambs

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One of the main sources of protein for feeding in intensive ruminant systems is soybean meal, however, they have a large environmental impact due to the use of land and water for large-scale cultivation. The use of insects such as *Tenebrio molitor* (TM) as a source of protein in ruminant feed could be a good feeding alternative. The objective of this study was to evaluate the effect of soya bean meal (SBM), fish meal (FM) or TM meal supplementation on animal performance in growing lambs. 24 lambs (LW 34.6±3 kg) were distributed randomised (8 lambs / treatment) to one of three isoprotein and isoenergetic diets (15% CP; 11 MJ ME / kg DM). The protein source was the only factor of diet variation. The control diet (SBM) included 150 g of SBM / kg DM, which was replaced by FM (50 g/kg DM) or TM (60 g/kg DM) meal in the other diets. Experiment duration was 28 d, 21 d for diet adaptation and the last 7 d for sampling period, Live weight (kg), Intake (g/d), digestibility (g/kg) and N balance (g/d) were recorded. Data were analysed for a completely randomised design; significant effects were declared at $P<0.05$. Dry matter (DM) and organic matter (OM) intake (1,387±60 and 1,309±50) were similar among treatments ($P>0.05$), DM and OM digestibility (760 and 763 g/kg respectively) was higher for SBM than FM and TM diets (731±2 and 739±4 g/kg respectively). N intake was similar (43±4 g/d) among treatments ($P>0.05$), N retention was higher ($P<0.05$) for SBM diet (14.05 gN/d) than FM and TM diets (10.64±1.2 gN/d).

Black soldier fly lipids in pet nutrition

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Black soldier fly (BSF) derived ingredients are being considered as sustainable alternatives to conventional animal derived ingredients in pet food formulations. However, BSF fat has received little to no attention from pet food producers until now. This work compiles the latest scientific knowledge on BSF fat on pet food applications. BSF fat digestibility, antimicrobial activity and brain health improvement potential have been analysed. Literature was only available on the use of BSF fat for dog pet food formulations. Outcomes indicate that BSF fat is highly digestible when compared with other fats used in pet food. BSF fat is rich in medium chain fatty acids and lauric acid, which are known for their brain health potential and antimicrobial activity, respectively. Studies on the functional benefits of these compounds have been evaluated in animal species with a similar metabolism to that of dogs and cats. Hence, this work suggests that similar benefits can be expected for other pets. Future studies should focus on verifying this hypothesis and filling the gap on BSF fat application in feline food formulations.

Sustainable mealworm rearing in China for insect ingredient produced for pets

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The pet industry has long been looking for new protein sources either for health improvement or for marketing needs, and the yellow mealworm (*Tenebrio molitor*) is currently the number one species being used in many aspects of the pet industry globally. YZ Pet Food has been growing yellow mealworms since 1998 and has developed a unique production system and product lines over the period. Here we want to demonstrate how a modern insect production facility functions from feed preparation to insect product processing.

Evaluation of black soldier fly larvae protein as substitute of poultry by-product meal in kibble diets for dogs

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Black soldier fly larvae proteins (BSFL) are gaining rapid acceptance as high value protein ingredients in pet food formulations. Moreover, some *in vitro* studies have indicated strong antioxidant and antimicrobial activities of BSFL. However, currently there are no *in vivo* reports to justify these findings. During this study, two isonutritive extruded diets for adult dogs (25% crude protein, 12% fat, 3.5% crude fibre, 5% ash) were formulated using two different protein sources: diet with 29% BSFL protein and diet with 28% low ash poultry by-product meal (PBP). Eight adult dogs were fed with formulated diets in a cross-over design with two periods of 60 days each. In this duration animals were subjected to faeces collection for digestibility and faecal parameters, skin barrier evaluation with suitable device and blood collection for biochemistry, antioxidant, and immunological assays. It was possible to extrude diets even on a high level of inclusion of BSFL, resulting in kibbles with acceptable density (380 g/l), proper cooking and expansion. Dogs presented good acceptance of diets, with no refusal episodes and well-formed stools, with similar faecal scores (of 3.7 and 3.9, on a 1 to 5 scale where 1 is watery faeces and 5 hard and dry faeces, 3 and 4 is considered ideal) and dry matter digestibility (of 81 and 80%) for PBP and BSFL diets, respectively ($P>0.05$). Urinary pH (6.7 and 6.6, $P=0.602$) and density (1.023 and 1.021, $P=0.959$) were similar for PBP and BSFL diets, respectively, and found in a normal range for dogs. After 30 days, these parameters were similar between groups. The preliminary data showed promising results. To conclude, BSFL seems a valid alternative to be used in extruded diets with no nutritional prejudices.

Community development programme on sustainable production of insects as feed to support animal sector in western Kenya

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The demand for protein for animal feeds is increasingly becoming inaccessible for local farmers due to dwindling stock, high cost of soybean and fish meals, reduced arable land and water requirement, and high environmental footprints. Therefore, this project aims to develop and scale black soldier fly (BSF) protein production as alternative sources to the major protein used in fish and poultry feeds through technologies and innovative research that are sustainable and environmentally benign. Bondo Community Development Programme and Protein Masters have partnered to develop the BSF larvae production on biowaste in the target area. To upscale this technology local and foreign Universities, research institution and private sector partners would collaborate to train smallholder farmers on production, substrate recipes formulation, safety quality of processed insect products and feed formulation for poultry and fish production within Lake Victoria Region. The insect farming process would generate additional value-added product (insect frass fertiliser) for improve soil health and crop yield for improved food and nutritional security among the vulnerable segment of the community. This project will address global challenges such as protein shortage, climate change, greenhouse gas emission, environmental degradation, and unemployment with huge social and economic benefits for the youths and women.

Insect protein as a base for starter feed in aquaculture

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Intensive development of aquaculture supposes creating, controlling and regulating the quality of fish habitat and nutrition. That allows to carry out purposeful prophylactic and therapeutic measures. Nevertheless, intensive fish farming does not remove problems of fish health, growth, development and survival, especially at the stage of fry. The main reasons for the death of fry: conditions of maintenance and transportation, diseases associated with weak immune system, mismatch of feed to the physiological needs of fry in nutrients and biochemically active substances. Thus, intensive development of aquaculture directly depends on effective starter feed. Hypothesis: 'Using the unique antibacterial, therapeutic and nutritional properties of the genus *Lucilia* and *Hermetia illucens* fly larvae cultivated on different kinds of biological feed substrates, combining them as a basic ingredient, to create an effective starter feed for aquaculture application'. The research is organised in the research laboratory of NPO 'Agro-Matic', Leningrad Region. Conditions for rearing of fly larvae of *Lucilia* and *Hermetia illucens* on different food substrates were created in the laboratory, and the necessary amount of Recirculating Aquaculture Systems (RAS) for keeping experimental and control groups of trout and African Clarias catfish fry was established. The following tasks are solved experimentally: (1) to determine the optimal formulation of a feeding substrate for rearing fly larvae of the genus *Lucilia* and *Hermetia illucens*; (2) to determine the optimal ingredient proportions from the combination of two types of dried fly larvae for starter feed; (3) to develop an optimal formulation of starter feed for trout and African Clarias catfish fry using a combination of protein from two species of fly larvae; (4) to determine the optimum rates of starter feed with fixing of main parameters of vital functions of fry in comparison with a control group using a starter feed of BioMar company (Denmark). It is planned to finish the research in March-April 2023 and state the results and conclusions in a number of scientific articles for specialised editions.

The influence of high doses of insect PAP from black soldier fly (*Hermetia illucens*) on slaughter performance of chicken

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The aim of this study was to evaluate the effect of insect PAP from *Hermetia illucens* on slaughter performance of chicken broilers. Ross 308 broiler chickens were utilised in this research. Birds (n=800) were divided into 4 food groups: H0%: standard feed and H50%, H75% and H100%: soya replaced with the *H. illucens* PAP. Replacing soybeans with *H. illucens* PAP adversely affected the digestive system of poultry. The research showed numerous changes in the histological structure of the stomach, liver and intestines. The severity of morphological changes varied considerably. No relationships were observed in the analysed morphometric parameters. The muscles of chickens fed with insect PAP were characterised by a lower proportion of minerals, increased content of SFA and decreased of PUFA in IMF. The changes in the IMF fatty acid profile resulted in deterioration of the fat nutritional value indicators. Changes correlated with amount of PAP share in diet. In H50% group, the lowest level of autooxidative changes was found. Meat from groups H75% and H100% was characterised by a significantly lower juiciness and intensity of flavour, and a tendency to lower the desirability of flavour compared to H0% and H50%. The results indicate, that the slaughter values of broiler chickens fed with *H. illucens* PAP should be further analysed at the levels not exceeding 50%. Project financially co-supported by: NCBiR 2018-2021 Strategy for Using Insects as Alternative Protein Sources for Animal Feed and Prospects for Future Production in Poland: Minister of Science and Higher Education in the range of the program entitled 'Regional initiative of Excellence' for the years 2019-2022, Project No. 010/RID/2018/19, amount of funding 12.000.000 PLN.; NCBiR GOSPOSTRATEG1/385141/16/NCBR/2018; and 'Sustainable up-cycling of agricultural residues: modular cascading waste conversion system', supported by NCBiR research grant agreement No. FACCE SURPLUS/III/UpWaste/02/2020

Black soldier fly larvae fat affects the microecosystem of the broiler chicken gastrointestinal tract

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This study aimed to determine the effect of *Hermetia illucens* larvae (BSFL) fat added to broiler diets as a partial or total replacement for soybean oil on a gastrointestinal tract (GIT) selected microbial population and its activity. In total, 576 one-day-old female Ross 308 chicks were randomly allotted to 3 dietary treatments (16 replicates and 12 birds each). Birds had *ad libitum* access to water and feed for 35 d. The following groups were applied: SO – 100% soybean oil, BSFL50 – a mixture of BSFL and soybean oils in a 50:50 ratio, BSFL100 – 100% BSFL fat. The digesta samples from the crop, jejunum, and ceca were analysed using fluorescent *in situ* hybridisation, and gas chromatography. The application of BSFL fat in broilers' diets resulted in increased proliferation of potentially pathogenic bacterial populations in the crop. Furthermore, BSFL100 enhanced microbial activity via total SCFA production and lowered the pH in this segment. The strongest impact on the reduction of select components of the microbial population in the cecum was observed with the BSFL50 treatment for potentially pathogenic bacteria such as Enterobacteriaceae, *Bacteroides* – *Prevotella* cluster, while commensal populations were also limited, i.e. *Bacillus* spp., *C. leptum* subgroup, and *C. coccoides* – *Eubacterium rectale* cluster. The present study revealed that BSFL fat led to adverse microbiota shifts in the chicken crop because of insufficient release of lauric acid. Nevertheless, the neutral effect of BSFL fat administration on the chicken jejunal microbiota and its activity or positive influence on cecal bacterial population content were noted. This work was supported by National Science Center project no. 2020/39/B/NZ9/00237 titled 'The role of *Hermetia illucens* larvae fat in poultry nutrition – from the nutritive value to the health status of broiler chickens'.

Sensorial evaluation of breast of chicken reared in organic system and supplemented with live black soldier fly larvae

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Live larvae fed to poultry has shown to provide good nutrients and bioactive compounds with positive effects on bird's health. However, trials on chicken reared for meat consumption are still scarce. A total of 240 Label naked neck (LNN) birds were reared from 21 to 82 days of age, and four experimental groups (10 birds/pen, 6 replicates/treatment) were considered according to the birds' gender and larvae provision. Experimental groups were fed 10% supplementation of black soldier fly (BSF) live larvae based on the daily feed intake. Birds (12/diet, 2 birds/pen) were slaughtered according to the standard EU regulations. Following storage at 4 °C for 24 h, breast fillets were excised, vacuum packaged and transported to the laboratory for analysis. Vacuum packed breasts were cooked in a water bath at 75 °C for 45 min, then breast colour and drip loss were measured. Sensory descriptive analysis was performed, in duplicate, by 11 trained judges, experts in sensory evaluation with a specific software for sensory data acquisition, (FIZZ Biosystèmes), using a nine points intensity scale. Results were elaborated by a statistical analysis using R software. Colour measurement, drip loss percentage and sensorial profiles were analysed through the ANOVA and post hoc test (Tukey's HSD). No significant differences were found in mean percentages of drip loss while colour measurement showed only differences based on chicken gender and only for the b* (yellowness) parameter that resulted higher in females. Concerning sensory evaluation, no significant differences were found. In conclusion, results highlighted that a dietary 10% supplementation of BSF live larvae did not affected sensorial quality of breast fillets of LNN chickens reared in an organic production system. The authors acknowledge the financial support for Poultryinsect project provided by transnational funding bodies under the Joint SUSFOOD2/CORE Organic Call 2019.

Rheology and consumer acceptability of cricket (*Acheta domesticus*) enriched chapatti in Bangladesh

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Edible insects perfectly fit into the UN Sustainable Development Goals. They have an excellent nutritional profile and are also considered as sustainable food sources. However, in several countries, insects are not (yet) on the daily menu, and therefore invisible incorporation in a traditionally consumed food product is required. More specifically, chapatti is an important part of the daily menu in Bangladesh, and it is thus a good study object for enrichment with cricket powder (*Acheta domesticus*) to improve the food security in the country. In previous research, it was shown that supplementation of 5% cricket powder in wheat flour produced a dough and chapatti comparable with a reference chapatti in terms of rheological and textural properties. An addition of 10% cricket powder had a noticeable impact on the properties of the dough and baked chapatti. Further rheological improvement of dough and chapatti using different hydrocolloids was studied using a Design of Experiments. Based on the dough properties, a combination of carboxymethylcellulose, hydroxypropylmethylcellulose and guar gum was selected as optimal composition for a chapatti with 10% cricket powder. This recipe was also successfully validated. To determine the sensory acceptability, four different types of chapatti: (1) white and; (2) whole wheat flour, and white wheat flour enriched with (3) 5% and (4) 10% cricket powder including the selected hydrocolloids] were evaluated in Bangladesh by a trained panel for 9 parameters (e.g. colour, puffiness, strength, pliability, taste, etc.) and by a consumer panel (like/dislike). For all 9 parameters, the trained panel scored the chapatti with white wheat flour as the best product, closely followed by the chapatti enriched with 5% cricket powder as the second-best option. Also, the consumer panel liked the chapatti with 5% enrichment. This research therefore shows that chapatti enriched with crickets is a promising product for an improved food security in Bangladesh.

Effects of *Hermetia illucens* live larvae as environmental enrichment on poultry gut health

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Hermetia illucens (HI) is a source of nutrients for poultry, both as live larvae and meal. Even if chitin (present in both HI forms) has been reported to negatively impact diet digestibility, it may also have beneficial effects on gut health. This study aimed to assess the effects of HI live larvae on the gut histomorphology of intermediate-growing chickens. Male chickens of 14 days of age (n=84, Label Naked Neck) were allotted to three dietary treatments (4 pens/treatment, 7 chicken/pen): control (C) fed a commercial diet; HI10 and HI20 (C + 10 and 20% of the bird expected daily feed intake of HI live larvae, respectively). In all the treatments, the C diet was distributed *ad libitum*. At day 82, 16 birds/treatment (4 birds/replicate) were slaughtered. Duodenum, jejunum, ileum, liver and Bursa of Fabricius were sampled and submitted to morphometric (villus height-Vh, crypt depth-Cd, villus width-Vw, Vh/Cd ratio, total absorptive area, and intestinal mucosa and muscular thickness) and histopathological analyses. Data were analysed by R software. The Vh, Cd, mucosal thickness and the total absorptive area were lower in HI20 than C and/or HI10 groups ($P<0.05$). Moreover, Vh, Vh/Cd, Vw, mucosa thickness and the total absorptive area decreased from duodenum to jejunum and/or ileum while muscular thickness increased proximo-distally ($P<0.05$). The diet did not influence the severity and distribution of inflammation in liver and cortical depletion of Bursa of Fabricius ($P>0.05$). However, the duodenum of HI20 group showed less inflammation than that of the other groups. In conclusion, despite the potential negative reduction in gut absorption surface of HI20 birds, the reduction in duodenum inflammation, as well as the overall maintenance of physiological gut development, suggest that HI live larvae can guarantee animal's gut health. The CELLOWFEEP research received the support of Fondazione Cariplo (Italy).

Inclusion of live black soldier fly larvae in poultry diet: impact on growth performance

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The use of insects in animal feed has the potential to reduce the demand for soybean production and to reduce the deforestation and the loss of natural resources. In particular, the black soldier fly (*Hermetia illucens*, HI) larvae have received great attention due to their ability to convert organic waste into high-value biomass and to provide high quality nutrients for monogastric animals. The aim of this study is to evaluate the effects of the inclusion of HI live larvae in the diets of chickens on their growth performance. Male slow-growing chickens (n=84, Label Naked Neck) of 14 days of age were randomly allotted to 12 pens. Each pen was randomly assigned to a dietary treatment (4 pens/treatment, 7 chickens/pen) as follows: (1) control diet (C), commercial feed; (2) HI10, C + HI live larvae (g) corresponding to 10% of the expected daily feed intake (DFI); and (3) HI20, C + HI live larvae (g) corresponding to 20% of DFI. The commercial diet was distributed *ad libitum* in all the treatments. At 82 days of age, the chickens were slaughtered. Analysis of variance was performed using the software SAS 9.4. All growth performance parameters (body weight, feed intake, feed conversion ratio) were significantly affected by the dietary treatment and age ($P<0.0001$). The diet $\times$ age interaction significantly ($P<0.0238$) affected feed conversion ratio, with the lowest value recorded in the HI20 treatment from 21 to 82 days of age. The diet significantly affected the slaughter performance parameters ($P<0.05$): the lowest values were recorded in the C treatment, whereas no difference was recorded between HI10 e HI20. In conclusion, growth and slaughter performances were positively affected by the administration of HI live larvae, even if with some differences between HI10 and HI20 treatment. The CELLOWFEEP research (Project n. 2019-1944) received the support of Fondazione Cariplo (Italy).

Inclusion of black soldier fly meal (*Hermetia illucens*) fed with different substrates in feed for European Seabass

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In pursuit of the sustainability of aquaculture, a key aspect is alternative protein sources that substitute fishmeal. Several studies have shown that black soldier fly (*Hermetia illucens*) meal could substitute a significant amount of conventional sources of protein in aquafeeds. However, because the composition of the larvae varies according to their diet, the performance of the fish when including them in the feed may be different depending on the culture substrate of the larvae. 192 European seabass (*Dicentrarchus labrax*) (initial body weight 180 ± 0.2 g) were weighed and divided into 12 cylindrical-conical tanks. Four diets were formulated (3 replicates/diet), a control diet with fishmeal and three diets containing full fat meal from black soldier fly larvae fed on different substrates (vegetables, brewery spent grains or whey) in substitution of 45% of the fishmeal. Fish were fed 7 days a week until apparent satiety. The feeding trial lasted 42 days. For each tank, weight gain, feed intake, specific growth rate, feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated. Hepatosomatic (HSI) and mesenteric fat (MSI) indexes were also evaluated. Fish fed diet containing vegetable-fed fly larvae meal showed lower growth and higher FCR, as well as lower PER, compared to the control diet. This could be due to a lower efficiency in the use of proteins with the diet containing vegetables-fed fly larvae meal. The other diets showed similar performance parameters to the control diet with fishmeal. The HSI of fish fed insect diets was lower than that of the control diet, which can be explained by the fatty acid profile of the insects. The performance and nutrient utilisation of the fish are affected by the type of substrate with which the insect larvae included in the feed are fed. These results are of great importance both for the aquaculture industry and for the breeding of insects as food for fish.

Inclusion of complete or reduced-chitin black soldier fly meal (*Hermetia illucens*) in feed for European Seabass

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An issue often reported when using insect meals in fish feed is their high chitin content. Chitin is a high value product that can be used for a wide variety of applications. Chitin is the main component of the exoskeletons of insects and is poorly digestible by fish. Therefore, removing chitin could improve its use in fish feed. A total of 192 European seabass (*Dicentrarchus labrax*) (mean initial body weight 180 ± 0.2 g) were weighed and divided into 9 cylindrical-conical tanks. Three diets were formulated (3 repetitions/diet), a control diet with fishmeal and two diets with black soldier fly larvae meal (one with complete meal and the other with reduced-chitin meal), replacing 45% of the fishmeal. Fish were fed 7 days a week to apparent satiety and uneaten feed was recovered. The feeding trial lasted 42 days. For each tank, weight gain, feed intake, specific growth rate, feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated. Whole body composition of the fish was analysed. Hepatosomatic (HSI) and mesenteric fat indices were also evaluated. The fish-fed diet containing reduced-chitin fly larvae meal showed a better utilisation of the diet (FCR and PER similar to the control diet). While the diet with complete meal showed higher FCR and lower PER. This could be due to a better use of proteins in the reduced-chitin diet. The fat content of the fish was lower with the diet containing reduced chitin meal. The HSI of fish fed insect diets was lower than that of the control diet, which can be explained by the fatty acid profile of the insects. The removal of chitin from insect meals can increase the quality of the insect protein and thus improve diet performance in sea bass.

Formulating and characterisation of local enteral foods in Cameroon enriched with *Macrotermes subhyalinus*

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This study aims to improve the nutritional value of some local cereals (*Sorghum bicolor*, *Zea mays* and *Oryza*) and termites (*Macrotermes subhyalinus*) for enteral nutrition. A mixing plan was established to take into account a specification allowing the formulation of 18 (eighteen) liquid diets. As well, the cereals used (rice, corn, sorghum) were soaked for 24 and 48 hours respectively, dried, crushed and calibrated. Subsequently, all the formulations were characterised in order to characterise their nutritional properties. Furthermore, two ratios (1/15) and (1/20) were used for the dilution and cooking of the formulated flours. Of the various enteral solutions obtained, some rheological analyses were carried out. This work revealed that, the chemical composition of *M. subhyalinus* concerning water content, ash, lipids, proteins and carbohydrates are respectively 6.96; 3.47; 43.12; 48.75; 4.66 g/100 g DM and their energy value 601.44 kcal. The protein and lipid contents of the formulated flours increased proportionally with the rate of termites incorporated. In contrary, carbohydrates decreased when termites were incorporated. The water content of the various liquid formulations varied from 8.911 to 11.97 g/100 g DM. Ash content varied from 7.29 to 13.10 g / 100 g DM. Energy value varied from 461.86 to 506.28 kcal. The viscosity and the pH varied respectively from 54.96 to 499.5 $\mu\text{Pa.s}$ and from 4.71 to 6.71 for the ratio 1/15, and from 23.5 to 399.667 $\mu\text{Pa.s}$ and from 4.89 to 6.713 for the 1/20 ratio. This work showed that the enrichment of composite cereals flours with termites (*M. subhyalinus*) increase significantly their nutritional value. These liquid mixtures can be used in enteral nutrition, or in order to fight against undernutrition in a hospital environment.

Effect of the rate of incorporation of insect powder (*Gryllus campestris*) on the feed value of pellets for fish

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Because of the entire importation of fish pellets in most African countries as Cameroon, we carried out this work whose aim is the formulation and characterisation of insect-based fish pellets. The main objective of this study is to contribute to the development of fish farming by producing cheaper fish pellets containing the highest rate of proteins. To achieve this, we have established a mixing plan from the Expert Design software which generated twenty diets formulae. For those diets formulas, the levels of cricket meal varied from 0, 0.62, 0.85, 1.25, 1.67, to 2.5 g per 25 g of pellet weight, depending of the quantity of the others ingredients used as cassava flour, corn bran, wheat feed, peanut cake and cotton seed cake. The nutritional values of pellets obtained and some rheological analyses as density, friability, hardness, buoyancy index and mass of 100 pellets have been performed. From this methodological approach, it appears that the protein content, lipid, ash and dry matter crickets are respectively 62.6 g/100 g MS, 9.6 g/100 g MS, 9.8 g/100 g MS and 94.3%. The nutritional value of diets varies respectively from 85.19 to 95.72% for dry matter 2.82 to 6.79 g/100 g MS for ash content 2.18 to 4.00 g/100 g MS for the content of lipid and 9.96 to 22.88 g/100 g MS for protein content. Then we performed 67.52 to 84.00 g/100 g MS for the carbohydrate content and 408.45 to 388.66 kcal for the energy value. The rheological analyses done allowed identifying 7 samples. The friability varies between 24.50 to 35.29%. At the end of this work, it emerges the fish pellets formulated based *Gryllus campestris* can be used to improve the productivity of aquaculture using local resources.

Insect production systems/genetics, nutrition, physiology, health & ethics

Influence of strain genetics on larval performance and bioconversion efficiency for *Hermetia illucens*

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Due to increasing welfare and population, demands for more sustainable protein sources are rising in today's society. Insects are considered such an alternative as they have short life cycles, high feed conversion and can be grown on low-value feedstocks. Particularly the black soldier fly *H. illucens* is able to convert low-value organic side streams into high-value biomass composed of proteins, lipids and chitin. Therefore, *H. illucens* larvae can be used for waste reduction paired with the production of high-value biomass, bringing more circularity in our food- and agricultural industry. Although the black soldier fly has been subject of extensive research and suggested as the crown jewel of an emerging insect-livestock sector, characterisations of its genetic resources, crucial for future breeding progress, have been neglected so far. Recent studies using wild and captive strains demonstrate that there is remarkable genetic variation across origins, including signatures of domestication. However, it still remains to be elucidated how genetic differentiation may translate into distinct phenotypic traits, such as economically interesting larval performance and bioconversion. In this study 10 captive *H. illucens* strains were obtained and reared using a standardised protocol. The strains were genotyped based on the 15 microsatellite markers developed by Kaya *et al.* Subsequently, larvae were reared on 3 different diets and larval performance and conversion efficiency was calculated. This allowed to investigate the influence and potential interactions of genotype and diet on these economically interesting traits.

Can we ensure a humane death for black soldier fly larvae used in food and feed?

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The International Platform of Insects for Food and Feed (IPIFF) states that 'We are committed to promoting good welfare practices in husbandry, transport and at the point of death, caring for insects' well-being.' Good welfare at the point of death means a death that is free from pain or suffering, which can be achieved by killing the animal instantly. Blanching is often cited as a method to kill black soldier fly larvae (BSFL) instantly, and yet it appears that there is no empirical data to support this. This study piloted methodology to answer the question: what is the lowest temperature of water needed to cause instantaneous death in black soldier fly larvae. Instantaneous was defined as within one second, and death was defined as lack of response to mechanical stimulation and loss of turgidity. Results indicated that the proxy measures used to determine death need further refinement, in part through a clearer understanding as to whether post-mortem reflex activity can occur in BSFL. Through presenting this pilot work we wish to stimulate discussion and collaboration on the important topic of humane killing of insects used in food and feed, so that insect farming companies can meet the growing societal expectation that insects, as living creatures, are treated with care and respect.

Influence of larval density on estimated digestibility of a standard diet in black soldier fly (*Hermetia illucens*)

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Black soldier fly larvae (BSFL) efficiency at converting organic by-products into insect biomass suitable for livestock nutrition depends on the nature and macronutrient profile of their feed. Digestibility is a key parameter to the development of a diet formulation approach, but methods to assess digestibility in most insects are unsuitable for BSFL as ingesta is not measurable and pure faeces can not be obtained and analysed. This work defined estimated digestibility (ED) as the difference between feed and frass nutrient weight, divided by the weight of nutrient in feed, and explored its variation with larval density. ED of dry matter (DM), starch, nitrogen, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ash and energy were evaluated using the same amount of chicken feed, same feeding time and densities from 0 to 29 larvae/cm². ED of DM, starch, nitrogen, EE and energy increased with larval density following an asymptotic trend. ED of ash also increased between 0 and 11.4 larvae/cm², but decreased for higher densities. For NDF, ADF and ADL, no clear results were obtained presumably because the fibre dosing procedure used was not specific enough and also accounted for chitin in the frass. ED of all nutrients but fibre fractions were modelled using a logistic equation. This method was able to propose asymptotic ED values for some major nutrients. This new indicator corresponds to the maximal fraction of each nutrient that BSFL can digest and shows potential for the development of a BSFL diet formulation approach based on digestible fractions rather than crude nutrient content. Future studies should compare asymptotic ED of various substrates to provide insight on BSFL ability to digest different ingredients.

Metatranscriptomics of black soldier fly (Diptera: Stratiomyidae) larvae during host feeding and fasting

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Insects and their associated microbes have long co-evolved, but little is known of insect-microbe interactions during variable conditions, including metabolic stress. Black soldier fly larvae represent a great model for investigating this relationship due to their lifestyle associated with complex microbial activities in extreme environments. Furthermore, this information will be greatly appreciated by the industry of mass-producing black soldier fly larvae as a potential alternative to increase protein production efficiency. In this study, we employed metatranscriptomic shotgun sequencing to investigate larval host starvation-induced functional shifts of the associated larval microbes. Black soldier fly larvae were fed standard diet or were under fasting conditions for up to 48 hours where their microbes were then processed through RNASeq and subsequent analyses. We discovered 104 prokaryotic genes that were expressed significantly ($q < 0.05$) between the starved and feeding cohort. Among these, 72 were down-regulated in the starved treatment group, most being associated with metabolism and information processing. The remaining 32 were up-regulated with approximately 20% of those being characterised as enzyme related or associated with transport systems. Our study demonstrated that metabolic stress induced by black soldier fly larval fasting had a great impact on the associated functional bacterial response. Further, our results suggest a system that larval microbes use to adjust to limited nutrients and adjust their metabolisms accordingly, thus benefiting individual species, potentially other bacteria through cross-feeding, and conceivably, the host, who also obtains caloric intake through microbial metabolism.

The role of high-energy diets in growth priming of the black soldier fly larvae

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Black soldier fly larvae (BSFL, *Hermetia illucens*) are known to be one of the best upcyclers of nutrients from organic waste into high-quality protein meals used in animal, poultry and aqua feed. In contrast to conventional livestock rearing, BSFL production facilities integrate broodstock rearing along with conventional 'grow-out' of larvae for processing. Key to an efficient broodstock system is the nursery stage in which first instar larvae (neonates) are reared on nutrient dense feeds during the first 3-6 days of their lifecycle. Although methodologies around Nursery differ between producers, it is consistently one of the most delicate stages and largely determines the success of the later bioconversion process. We conducted a study to evaluate the role of nutrition priming using high energy diets for neonates and the outcome of this through to the pupae stage. The study evaluated the impact of using sugar syrup (T1) and Molasses (T2) in the nursery stage feed compared to our conventional starch formulated diets, on the overall performance of pupae. The pupae rearing diet was identical for all treatments. At the nursery stage, the results showed that the high-energy diet boosted larvae activity and produced larger larvae ($P < 0.05$) when compared to the control. At the pupae stage, there were significant results in pupae yield, survival rate, and feed conversion rate as compared to Control ($P < 0.05$). Overall, we show that the BSFL fed on the high-energy diets and hence ingested sugars performed better on the later grow-out stage, despite the initial diets representing a low proportion of total feed intake. This impact may be partially explained by phenotypic plasticity, where nutritional cues in early life stages lead to lasting physiological changes, such as energy and development trade-offs. Phenotypic plasticity as an adaptation to the variable resources experienced by saprophagous species has been observed in Diptera with comparable life-histories such as *Drosophila melanogaster* and *Anopheles darlingi*. Further research on the impact of early stage feeding of BSFL is needed in order to understand how nutrition impacts life-history trade-offs in this important species.

A full sib design is a practically feasible way to estimate genetic parameters in black soldier fly (*Hermetia illucens*)

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Black soldier fly (BSF, *Hermetia illucens*) is recognised as an organism that upcycle organic by-products into sustainable ingredients used in feed for pigs, chickens, fish and pets. Unlike in larger livestock, BSF breeding program has not been widely investigated. One limitation is that BSF larvae are difficult to track individually over time, which makes it difficult to collect suitable datasets. The aim of this study was to design a scheme to estimate genetic parameters of BSF, collect the data, and estimate heritability's and phenotypic correlations between the recorded traits. A full sib family design was implemented in a practically feasible way to record multiple traits on the same individuals across a number of families. Genetic parameters were estimated using a linear mixed model. In total six traits were measured from 2,731 larvae, of which 1,285 were followed until emergence to adulthood. Heritability estimates for body weight at prepupa, body weight at pupa, development time to prepupa was 0.25, 0.45 and 0.38, respectively. The common environmental effect was large at the young larval stage and reduced after housing the BSF individually. In general, body weight showed a positive phenotypic correlation at the different life stages and development time was positively correlated with body weight at (pre)pupa stage. In conclusion, a full sib family design is a practically feasible way to collect data for estimation of genetic parameters in the BSF.

De novo transcriptome sequencing and analysis of *Ruspolia differens* midgut varies with diet and sex

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Ruspolia differens is a commercially important edible insect across Sub-Saharan Africa. Although much research on its nutritional quality has been conducted, the molecular mechanisms regulating diet preference in *R. differens* remain elusive. Also, the sex-dependence of gene expression repertoires in the midgut remains unknown. Transcriptomic analysis tools are needed to identify the vital regulators involved in digestion, immune defence, and detoxification mechanisms amongst male and female *R. differens*. In this context, we performed RNA sequencing analysis of the messenger RNA (mRNA) content of midguts from insects of different sexes and fed different diets. The RNA-Seq data were used to create an *R. differens* midgut reference transcriptome. Furthermore, differential expression analysis was performed to investigate differences in the midgut transcript profiles of males and females fed high protein, high carbohydrate, and control diets. A total of 21,992,013 reads generated by Illumina (NextSeq 500) mRNA sequencing analysis were assembled into 23,917 transcripts. Upon assembly, 8,398 unigenes were detected; the average length of unigenes is 1,064 nt, N50 is 1,312 nt. A total of 28 and 41 transcripts were significantly up- and down-regulated in the high protein compared to the high carbohydrate condition, respectively. Additionally, 31 and 34 were significantly up- and down-regulated between the sexes. The specific unigenes encoding known enzymes involved in digestion, nutrient transport, and detoxification were identified. This analysis demonstrated *R. differens*' ability to induce the expression of an array of genes in response to diets differing in certain food classes. This study provides a valuable transcriptome database to study further the digestive process and the physiology of the midgut in this insect. It, therefore, improves our fundamental understanding of this insect's midgut functioning and gives insight into genetic factors mediating diet-dependent changes in male and female *R. differens*. This is the first study of transcriptome sequencing and analysis in a *Ruspolia* species.

Black soldier pupae size – key to productivity & performance?

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The black soldier fly (BSF, *Hermetia illucens*) is currently increasingly utilised in industrial settings for its bioconversion ability and application as an animal feed ingredient. Industrial-scale BSF production requires the maintenance of large breeding populations, where variations in insect fecundity and fertility can have significant downstream impacts. This study examines how pupae weight can impact reproductive efficiency through the lens of adult fly mating behaviour. We produced and categorised the pupae into two groups: Large Pupae (0.18 g) and Small Pupae (0.12 g). Then we paired the adults into three groups: Large Pairs (Large Female × Large Male), Small Pairs (Small Female × Small Male), and Mismatch Pairs (Large Female × Small Male). The Large Pairs produce the highest eggs ($P < 0.05$) and neonates ($P < 0.05$), with the highest hatching rates ($P > 0.05$) recorded among all groups. The Small Pairs resulted in a much lower total production, but their hatching rates are at par with the Large Pairs. While the Mismatch Pairs have generally lower productivity ($P > 0.05$) and hatching rates ($P > 0.05$) compared to the Large Pairs. Our visual observation of each pairing group suggests that pairing sizes influence mating behaviours. The males in Large Pairs and Small Pairs groups were able to grasp the females securely while the females were not very aggressive, suggesting that same size pairings have higher compatibility. On the contrary, in the Mismatch Pairs group, the larger females and small males had poor compatibility during mating, and the grasping ability of the smaller adult males was challenged by their larger partner. In order to optimise the use of broodstock resources, BSFL producers should consider maximising the individual pupae size while also taking steps to improve size homogeneity.

Social distancing: assessing the optimal 3D mealworm density

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Any efficient farm wants to produce as much as possible high quality product on a certain area. This is true for both plants (e.g. perfect sowing distance) and animals (number of poultry in a shed). Insect farms are no exception to this rule. Yet, for some insect farms there is the additional difficulty that the larvae, partially, live inside their feed and we need to assess their density in a 3D space, not just the surface area covered. In this study we used *Tenebrio molitor* larvae as a model organism. An initial study indicated that changing the number of larvae per cm² (0.6–10) has no effect on their growth as long as the available volume per larvae remained identical (1 larvae/cm³). In this follow-up experiment, the optimal volumetric density was assessed. This was done in a pilot scale test at Inagro's Insect Research Centre with nearly 2 million 4 week old larvae. They were distributed in 80 crates with between 1000 and 128,000 larvae per crate. The volumetric density ranged between 0.5 and 8 larvae/cm³ and overall feed volume between 2 and 16 l (in a 60×40 cm crate). The feed used was wheat bran with a particle size below 2 mm and was replenished to the original volume at least once a week. The growth of the larvae was assessed each week and the core temperature was continuously monitored via in-crate sensors to detect deviations from the ambient temperature. The initial results indicate that mealworm larvae perform best at a low density of 0.5 to 1 larvae/cm³ and a high volume of feed (8 to 16 litres). For example, the growth decreased by 10–15% at week 8 when we compare 0.5 vs 8 larvae/cm³ and the growth increased 30–60% from a 2 l volume to 16 l. In part performance coincides with the actual core temperature of a crate. With a better growth with a core temperature of ±30 °C (instead of the 27 °C ambient temperature) but reduced growth when the temperature exceeds 35 °C.

Dose-response effects of starch in chicken excreta on *Musca domestica* larval performance and bioconversion efficiency

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Housefly (*Musca domestica* L.) larvae can convert animal manures into valuable protein and fat. Bioconversion of manure might be limited by energy available to the larvae or microbiota present in the manure. In this study we assessed the effects of differential starch content in unsterilised (UE) and heat sterilised chicken excreta (SE) on larval performance and bioconversion. Gelatinised corn starch was added to excreta to construct substrates with 0 to 50% starch based on total substrate dry mass with 3 replicates per dose. Substrates were inoculated with housefly eggs (8/g wet substrate) and larvae harvested by floatation after 5 days. Individual larva wet mass ($n=30$ /replicate) increased from 12 ± 0.2 (UE) and 13 ± 0.4 (SE) mg at 0% starch up to 18 ± 0.4 (UE) and 18 ± 0.5 (SE) mg at 15% starch and decreased to 5 ± 0.4 (UE and SE) mg in 50% starch. The decrease at higher starch content was faster in UE than in SE. Highest total larva wet yield in UE was 39 ± 2.3 g at 10% starch, 43 ± 2.8 in SE at 15% starch, decreasing to a minimum of 3 ± 1.5 g (UE) and 4 ± 1.3 g (SE) in 50% starch. The decrease in yield was faster in UE compared to SE. Larval survival was highest in 0% starch, $80\pm8\%$ (UE) and $72\pm13\%$ (SE), decreasing gradually to $14\pm7\%$ (UE) and $18\pm3\%$ (SE) in 50% starch. Dry matter bioconversion increased from $4\pm0.1\%$ (UE) and $4\pm0.3\%$ (SE) in 0% starch to 6 ± 0.2 in UE with 10% starch and 7 ± 0.3 in SE with 15% starch, with minima of $0.2\pm0.0\%$ (UE) and $0.6\pm0.2\%$ (SE) in 50% starch. The highest nitrogen bioconversion was $15\pm0.5\%$ in UE with 10% starch and $15\pm0.5\%$ in SE with 15% starch, decreasing with higher starch content. These optimal starch inclusions to UE and SE increased nitrogen bioconversion with 40 and 100% respectively, compared to pure excreta. This indicates that adding easily digestible energy to chicken excreta can substantially increase larval yield and nitrogen bioconversion.

Adventurous or conservative: inbreeding or outbreeding preference and its consequences for *Tenebrio molitor*

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The potential for artificial selection with insects for food and feed is a massive but largely unexplored territory. Most of the work on genetics and selection has been done on laboratory model species like *Drosophila melanogaster*. Although this research vastly increased our basic knowledge on insect genetics and physiology, it is rarely studied with the explicit aim of improving the economic potential. For two species, the honey bee and silk moth, both academical and industrial research has been done to significantly improve the species towards a higher production of respectively honey and silk. In this study we used *Tenebrio molitor* as a model species for insects for food and feed. We assessed the growth speed, maximum size and reproductive potential of 4 potentially inbred parental strains (bred for >10 years without outside interference) and their hybrid offspring by mixing two parental populations (6 hybrid strains) on a pilot scale. The hybrids were created by mixing pupae of equal age from the different pure strains. However, because of the at random mixing, we first assessed the preference for in or out-breeding of the emerging beetles from the different strains. This was done by presenting either a two week old virgin male or female simultaneously with two virgin beetles of the opposite sex from the same strain or another strain. The initial results indicate that different strains have extremely wide different preferences and there are variations between the males and female choices, as some were more 'conservative' than others. As a result, the percentage of hybrids when two strains are mixed randomly may be different compared to true 'at random' copulation. This may therefore influence potential future genetic experiments and programs relying on mass hybridisation.

Significant heritability of larval lipid storage in the housefly and its implication for breeding programs

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Lipids are essential nutrients for many critical insect functions (flight, development, reproduction). These nutrients form a significant fraction of the nutritional profile of insects and the presence of commercially relevant fatty acids make insect larvae a sustainable source of these valuable products. Lipid accumulation takes place during the larval growth. The quantity of lipids can vary among individuals and this trait is highly variable. An excess of energy intake can lead to obese larvae. Still, it is unknown to what degree lipid storage is genetically determined and inherited. Such knowledge is essential to selectively breed favourable phenotypes for commercial purposes. Here we determined the heritability of larval lipid storage in the common housefly (*Musca domestica*). A nested mating design consisting of 47 full-sib families produced 663 larvae which were individually phenotyped for their total fat content. Rearing conditions and adult selection were strictly standardised to reduce environmental variation in lipid content among individuals. Individuals were separated based on their time to reach pupation and their emergence day, and subsequently pooled into randomised groups. Three statistical models were built to estimate the heritability of larval lipid accumulation a linear model (lme4) and two animal models: a Marchov Chain Monte Carlo generalised linear model and one based on restricted maximum likelihood. The two animal models show a moderate heritability for larval lipid storage whereas the linear model estimated a low heritability. Contrarily, heritability estimates for larval dry weight were low independent of the model used. This study provides a first glimpse on the quantitative genetics of a trait of high relevance for the emerging insects as feed and food sector. It also informs on how to set up a tailored breeding program to select strains with improved lipid characteristics.

Agar as a nutrient carrier: a means for targeted delivery of nutrients to mealworms?

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Insect diet impacts insect growth, therefore a lot of recent research has focused on the evaluation of alternative insect feeds. In the case of the yellow mealworm, *Tenebrio molitor* L., the diet comprises of both a dry feed and a wet feed. The latter serves mainly as a moisture source, as it has been shown that moisture availability enhances larval development. Agar can efficiently serve as a water source for *T. molitor*. It is also used as the gelling agent of artificial diets for several insect species, serving as the carrier of the diet nutritional components. However, the use of agar as a nutrient carrier for mealworms has not been evaluated yet. Hence, the objective of the study was to evaluate agar for the targeted delivery of nutrients to *T. molitor* larvae. In a first laboratory trial, groups of 50 freshly-hatched larvae were provided with wheat bran and agar (20 g/l; 1×1×1 cm cubes 3 times per week) with different concentrations of brewer's yeast (0, 0.5, 1, 2.5 and 5%) as wet feed. In two additional pilot-scale trials, approximately 10,000 larvae were reared in crates on wheat bran, providing agar with various concentrations of baker's yeast or white sugar (0, 2.5, 5 and 10%). The larval growth and performance was evaluated at weekly intervals. According to the results of the lab trial, the increase of the concentration of brewer's yeast in agar enhanced larval performance in terms of growth rate and larval weight. In contrast, the provision of high sugar agar (10%) negatively affected larval growth and reduced larval weight compared to control. Our study aims to highlight the potential of agar as nutrient carrier for the targeted delivery of nutrients to mealworms. This study was funded by the MIS 5045804 project under the 'Operational Programme Competitiveness, Entrepreneurship and Innovation – EPAnEK 2014-2020', Greece-EU.

Experimentally evolving lean houseflies: a novel approach to select for insect larvae with reduced lipid content

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Energy-rich diets induce obesity in insects negatively affecting their fitness. High larval lipid content can reduce their usability in commercial feeds. Therefore the industrial applicability could be improved if an insect strain with reduced fat storage were available. We report on the results of experimental evolution to evolve larval phenotypes with reduced lipid content in the housefly *Musca domestica* under three dietary regimes. The dietary treatments consisted of a control (standard artificial) diet (CTRL), a sucrose-rich diet (SUG), and a diet enriched with dairy fat (FAT), with 5 replicate lines ($\pm 2,000$ individuals each) per treatment. After 12 generations, we measured dry weight, absolute lipid content and relative lipid content of the larvae from day 4 till pupation. In this generation, all lines were also reared on the control diet to test if differences in lipid content among the nutritionally challenged evolved lines were maintained when reverted back to the ancestral diet. Significant differences in lipid content were found among dietary treatments. When fed the experimental diet, the FAT treatment had significantly higher absolute and relative lipid content than both SUG and CTRL treatments. Effects were most pronounced in the early larval stage as the FAT larvae significantly reduced their relative lipid content towards pupation. These results suggest that the larvae converge towards a specific lipid content at pupation despite the high dietary lipid intake. When reverted to the standard diet, the SUG treatment showed a significantly lower absolute lipid content at pupation compared to the other two treatments. Treatments did not differ in relative lipid content. These results demonstrate that houseflies can evolve the ability to downregulate their lipid storage. We conclude that experimental evolution can be an effective tool for selecting phenotypes with commercially relevant characteristics. Our work also provides insights into the adaptability of the housefly to nutritionally challenging diets and opens new avenues for their application in commercial mass-rearing programs.

Young cricket production: separate, stabilise, then scale up

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Efficient insect reproduction is a core component of any successful insect edible farm. A farm will fail without reproduction, but mating, egg development, and oviposition all take time and resources without increasing the final economic value of the adult insects involved. At Aspire, we maximise the reproductive potential of an isolated breeder group while minimising the rearing time of all other insects, an approach which we find maximises efficiency and profit. Using this separate breeder population, we systematically conducted research to first develop essential procedures that ensured a steady production of enough young crickets ('pinheads') that met daily requirements, and then we scaled up production while still reducing cost per unit. This presentation will include results from a series of experiments beginning in 2016 that, when combined, allowed Aspire to first stabilise pinhead production and then increase production by 8-fold per mated cricket pair. This presentation will discuss many of the factors involved in cricket reproduction, including the following: methods for minimising procedural steps while still allowing for adequate population monitoring, the design of lay environments and hatchery management, and pest insect and microbe control.

The impact of diet on black soldier fly (Diptera: Stratiomyidae) thermal tolerance and preference is stage dependent

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Thermal tolerance and preference are traits commonly used to predict animal distribution and behaviour. In this study, impacts of diet composition on black soldier fly, *Hermetia illucens* L. (Diptera: Stratiomyidae) thermal tolerance and preference were examined. Black soldier fly larvae were offered either a low protein (7% ww) high carbohydrate (35% ww) diet (i.e. P₇C₃₅), a high protein (35% ww) low carbohydrate (7% ww) diet (i.e. P₃₅C₇), or the Gainesville diet as a control (~13% protein and ~32% carbohydrate). Results reveal that the black soldier fly thermal tolerance and preference were impacted by diet composition, while the impacts were stage dependent. The control diet resulted in the lowest heat tolerance in larvae and prepupae, but not in adults. The diet P₃₅C₇ resulted in the greatest heat tolerance in prepupae and female adults, but not in larvae. The shortest cold coma recovery time in larvae, prepupae, and adults were fed with diet P₃₅C₇, diet P₇C₃₅, and the control diet, respectively. Larvae fed with the control diet preferred the highest temperature, but no thermal preference differences were found in prepupae and adults among diets. Though the body weight did not contribute to the thermal tolerance significantly, data indicate a universal trend that individuals with greater body weights seemed tolerate better both in heat and cold regardless of the stage and treatment. The stage-dependent nutrient effect determined in this study reveals the complexity of nutrients – thermal traits interaction. Attention should be paid to larval diets when weighing one stage more than the other facing thermal heterogeneity.

Results of a genetic improvement program of *Hermetia illucens* for increased larval weight – industrial applications

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Black soldier fly (BSF; *Hermetia illucens*) industrial farming has increased significantly in the last ten years. Interest in the species arises from the ability of its larvae to convert low-grade organic materials into high-value food, feed and technical products. In addition to its use in aquaculture and pet food diets, the European Commission has recently authorised the use of insects derived processed animal proteins in monogastric livestock diets. At present, the demand for insect protein is higher than the supply, and the demand is expected to grow 50 times more by 2030 globally. There is a need to further improve the efficiency of production, to meet the increasing demands for proteins in the feed and food industries under limited resources. One means of increasing supply is selective breeding, which has been widely applied in plants and other animal species, but not yet reported in BSF. Indeed, to date, there have been no public research results reflecting a focused, multi-year selective breeding effort in BSF. In 2019, a genetic improvement program for increased larval weight of BSF larvae was started. We present the outcomes of this breeding program after 16 generations of selection. The performance of the selected body weight line was compared to the base population line over six experimental rounds under different environmental conditions. Under automated production settings, an average improvement of +39% in larval weight, +34% in wet crate yield, +26% in dry matter crate yield, +32% in crude protein per crate and +21% crude fat per crate was achieved in the selected line compared to the base population line. The selection carried out in the context of the genetic improvement program translated into improvements under industrial production settings. This research clearly demonstrates the potential contribution of selective breeding to improve efficiency when farming BSF.

Fat vs oil supplementation of insect feed: effect on mortality, insect growth and FA composition

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In this study the influence of oil supplementation in terms of lipid source and fat content was explored. *Tenebrio molitor* larvae was fed for 15 days with supplemented feed using 5 different lipid sources differing in fatty acid composition, physical state and melting points. The lipid supplementation level was 7, 10, 15 and 20%. A control with 4.5% of fat was included. In this control treatment no lipids were added. The selected lipids for supplementation were linseed oil, olive oil, palm oil, palm stearin and fully hydrogenated rapeseed oil (FHRS). Insect development was followed up in terms of weight gain and death rate. The effect of lipid supplementation on the insect tissue was assessed by total fat content and fatty acid composition. High death rates were observed in the feeding trials with $\geq 15\%$ oil content in the feed. However, fat supplementation did not affect death rate or weight gain. No significant differences in fat content were observed, except for FHRS (slight decrease). However, significant changes in FA composition, such as increases in relative contribution of myristic, stearic, palmitic, oleic, linoleic and linolenic acid were observed. The increase in FAs was directly related to the FA composition of the provided lipid source. The observed high death rates in the feeding trials containing high content of liquid oil were attributed to the state of the lipid (liquid vs solid). Whereby the oils dissolved the protective outer-layer of the skin damaging it and obstructed the stigmata of the insects. Therefore, the insect death is attributed to death by asphyxia. Confocal microscopy confirmed the presence of oil in the stigmata supporting our hypothesis. Lipid supplementation with fat sources at the highest level (20%) did not cause a difference in death rate or weight gain compared to the control. The results from this study could help in the formulation of insect feed, if ingredients with high-fat content are used.

Unnatural selection – consequences of the evolutionary selection occurring inside black soldier fly rearing facilities

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As humanity turns to alternative protein sources to fuel its population expansion, the protein-rich black soldier fly (BSF, *Hermetia illucens*) is increasingly being regarded as a livestock animal. Multiple companies are developing or operating industrial-scale BSF mass rearing facilities with the goal of generating insect proteins and fats from a diverse range of organic feedstocks. We consider the selective pressures that industrial environments place on BSF and highlight some of the challenges and opportunities this selection presents to the BSF industry. We provide examples of patterns of phenotypic divergence in three genetically isolated populations reared in three different facilities. However, more work needs to be done to isolate the corresponding genotypic changes at the population level, allowing us to discern whether these differences are due to environmentally mediated selection pressures, or an expression of phenotypic plasticity from within the larger population. We also adapt some lessons from traditional livestock rearing and review the literature to generate recommendations for the minimum effective population size to maintain evolutionary potential in BSF facilities. This potential is especially critical to buffer against stochastic events that could lead to population bottlenecks, or when BSF will experience significant changes to their environmental conditions or diet. Finally, we make procedural recommendations that may encourage the complementary optimisation of both economic and fitness traits through selection mechanisms. Overall, if a mass-rearing facility is started with enough genetic diversity and if care is taken to avoid subsequent bottlenecks or unintended selective pressures, evolutionary mechanisms can increase the economic output of the facility.

Revivable eggs by cryopreservation for insect production enhancement

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The production of *Hermetia illucens* (BSF) is centralised with each factory producing the entire fly lifecycle, maintaining on-site colonies to supply their production. However, reproduction of BSF in captivity requires high investments and profound biological knowledge. The number of eggs produced from a BSF colony also varies from week to week, and the larvae are known to be very fragile and prone to high mortality during the first five days of life. Therefore, current BSF factories must produce surplus eggs and neonates to maintain their planned production capacity. Some large-scale BSF producers are developing new genetic strains of BSF with enhanced traits. Without a reliable preservation system, specialised genetic strains may lose some of their traits due to e.g. colony collapses or diseases. RECIPE aims to develop innovative technologies for cryopreservation of eggs and suspension of neonates that can enhance and consolidate breeding sites and allow for the implementation of smarter and smaller rearing sites in the proximity of waste streams. During the presentation the main results of the project will be presented including: Optimisation of cryopreserved egg/suspended neonates and packaging: FreezeM has been developing and optimising the process of suspended neonates and the packaging. The focus was to optimise the density, decrease handling and increase survival rate. Validation of the cryopreserved egg/suspended neonates: the suspended neonates from FreezeM has been validated at DTI's facility in Denmark; focusing on validating the suspended neonates' survival rate after different transportation condition/time and shelf-life. Business model: Bühler has conducted a business evaluation of different production value chain including distance from breeding to rearing, size of facilities and shelf-life of suspended neonates.

Population genetics and domestication of the black soldier fly (*Hermetia illucens*)

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Insect farming is on the rise to becoming a sustainable and economically viable food and feed industry. The black soldier fly (BSF; *Hermetia illucens* L.) is poised as key species of interest due to its natural bioconversion properties. However, genetic screening and management is currently overlooked. Here, we present the first high coverage (22×) genome-wide assessment of a global sample of BSF individuals (n=54) belonging to both domesticated and wild lineages. We identify a pronounced population structure within BSF explained primarily by captivity status. Phylogenetic reconstruction reveals a deep divergence in BSF lineages. Patterns of introgression are strikingly absent between diverged lineages of wild and domesticated BSF. We also show that commercial activity is dominated by a particular lineage whilst wild populations harbour significant genetic diversity. Recent domestication has had a significant impact on shaping the genomic architecture of captive populations and has left signatures of selective sweeps throughout the genome. This study not only sheds light on the process of domestication but highlights the importance of genetic screening and the introduction of breeding programs to this novel industry.

Lifetime macronutrient intake of *Gryllobates sigillatus*: which protein to carbohydrate ratio maximises yield?

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Diet strongly impacts cricket life history traits. The relative availability of protein and carbohydrates, for example, can influence growth and body size. Most cricket farms use a generic cricket feed formulation, so one way to increase yield of mass-reared crickets is to determine how development time and body size at adulthood are optimised with diet. A 3:1 ratio of protein:carbohydrates (P:C) has maximised weight gain in *Gryllus veletis*, but different species have different nutrient requirements, and it is unclear whether a similar ratio would be ideal in a farmed species such as *Gryllobates sigillatus*. To test this, we individually reared hundreds of *G. sigillatus* from hatch on seven diets with different P:C (8:1, 5:1, 3:1, 1:1, 1:3, 1:5, 1:8) and measured survival, time to adulthood, adult body size, and lifetime food consumption. We used a nutritional geometry framework to determine how lifetime protein and carbohydrate intake affect life-history traits of *G. sigillatus*. Our results will contribute towards an optimal standard feed formulation required to maximise *G. sigillatus* production.

Reproductive success of black soldier fly exposed to different macronutrient diet profiles and population densities

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Fertility is a major factor for animal agriculture to measure reproductive success (RS). It can be impacted by colony management (i.e. population density during immature and mature stages), feed (nutritional diet profile), and the surrounding environmental parameters. The academic data on black soldier fly fertility is restricted to the number of hatched eggs, without considering its specifics such as sperm number, viability and, most important, motility. To fill this information gap, our research team aimed to investigate the impact of four different diet profiles: Four protein levels ranging from 12-21% crude protein, and in turn different carbohydrate concentrations; and two different larval densities: 1 and 4 larvae/cm² on sperm quality (viability and motility) and number. Three day-old virgin males were used in the study. Two trials were performed, with a total of 30 samples per trial. The results found will allow insect farmers to estimate the fertilising capacity of the colony and, consequently, implement strategies to enhance productivity and reduce cost without compromising safety and quality.

Developing protein libraries – a tale of unidentified proteins

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Protein identification can help us understand protein changes due to genetic or environmental differences; identify bioactive peptides with special health properties; or provide information to understand biochemical processes within an organism. Some protein libraries are well characterised (e.g. humans) but many species are limited. To do proteomics on these other species, protein libraries further up the taxonomy tree are used. Higher taxonomy levels contain replicate inputs of the same protein and in addition, need to be highly homologous to obtain confident protein identifications. For this work, Insects were collected either from the wild (grass grub, *Costelytra givenii*) or from laboratory cultures (brown headed leaf roller, *Ctenopseustis obliquana*) in New Zealand. The torso was removed to restrict the presence of microbiota in the sample. The heads and legs of grass grub and brown headed leaf roller larvae were prepared for sequencing of the mRNA. The mRNA data was assembled using Trinity and Soapdenovo-trans to produce a protein library. The sequences were blasted to provide protein names and redundancies were removed. Grass grub and brown headed leaf roller were prepared for proteomic analysis by extracting the proteins, digesting with trypsin and separation with LC-MS/MS (Ultimate 3000, Thermo Fisher and QTOF mass spectrometer, Bruker Daltonics). The spectra was compared to in-house and publicly available NCBI libraries using Peaks, Bioinformatics Solutions. The hypothesis was to increase the identification of proteins from endemic New Zealand insects for identification of bioactives. Brown headed leaf roller larvae had 97 protein identifications with the family tortricidae NCBI:txid7139 library whereas the in-house library identified 141 proteins. The grass grub library had 25 proteins identified using the Coleoptera library NCBI:txid7041 while the in-house library identified 253 proteins. The development of these libraries improved the number of identified proteins in these samples up to ten-fold. These libraries will be beneficial for future protein work on these species and other members of their taxonomic family and research to identify bioactives with potential human health benefits.

Evaluating *Tenebrio molitor*'s productivity with different wheat by-products diets for standardised nutritional assays

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In an effort to maximise the use of locally-sourced feed in insect farming, this experiment aimed at comparing the impact of different feed on the yield of *Tenebrio molitor* larval biomass and frass. In order to control for the variability of the staple wheat diet, two sources of wheat bran were compared (100% European and 100% Canadian wheat bran). To assess the upcycling potential, two diets with Canadian residual grains were introduced in the experiment (50% wheat bran + 50% white beer spent grain and 25% wheat bran + 50% white beer spent grain + 25% wheat germ). Agar was fed *ad libitum* to ensure hydration. A total of 5,500 four week old larvae were grown in 60 cm by 40 cm plastic trays in a controlled-environment room (27 °C, 60% humidity, 12 h light cycle), until pupation began (10% threshold). Each tray contained 2,100 g of the desired feed. This experiment showed that the origin of the wheat bran did not affect the average individual larvae biomass (133±3 mg for European and 137±3 mg for Canadian wheat), suggesting that locally sourced controls could suffice in international nutritional assays. The experiment also showed that the inclusion of spent grain or wheat germ decreased larvae biomass (116±3 mg for added spent grain and 125±3 mg for added spent grain + wheat germ), implying that incorporating different wheat by-products based on availability can significantly impact the growth of larvae. The nutritional value of the larvae (% protein, % lipids, % ash) and the physico-chemical characteristics of the frass (pH, electric conductivity, C:N ratio, and total N, P₂O₅, K₂O content) from the different treatments were compared. The results of this experiment have important significance for the advancement of optimal insect farming, the use of locally available feed for maximised insect productivity, and the development of standardised nutritional assays.

Evaluation of peal millet (*Cenchrus americanus*) as an alternative feed substrate for field cricket (*Gryllus campestris*)

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Production of crickets as human food and livestock feed is gaining momentum in many parts of Africa. However, availability of affordable food substrates remains a major constraint to many small scale cricket producers. This study investigated effects of different feed types on growth performance, feed conversion and nutritional composition of field crickets. The study was conducted at Chinhoyi University of Technology, Zimbabwe using five treatments (chicken mash plus potato, chicken mash, millet mash, millet mash plus potato and wheat bran as a control and replicated three times. The study showed feed related variation in growth, feed conversion, nutritional composition and survival rate. Significantly higher ($P<0.05$) survival rates were recorded on wheat bran (100%), and the lowest on millet mash (73.3%). The nutritional attributes of crickets on maturity were also dependent on the feed provided with individuals that were fed with 'chicken mash plus potato' having the highest feed conversion ratio of 3.1 g/g. Millet mash plus potato, wheat bran, chicken mash and millet mash treatments had 2.7, 2.6, 2.1 and 1.7 g/g, respectively. Correspondingly the 'chicken mash plus potato' treatment showed the highest amino acid content while crickets fed on millet mash only had the lowest amino acid content. There was a significant effect ($P<0.05$) of feed type on crude protein of crickets and results were in the order of: chicken mash plus potato>millet mash plus potato>control>chicken mash>millet mash. Our study showed great potential of millet mash as an alternative feed stream for cricket production. However, its performance has to be boosted by combining it with other feed ingredients such as root tubers.

Habitat-centric versus species-centric approaches to edible insects for food and feed

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The current paradigm of rearing insects for the food and feed industry uses a species-centric approach. With this approach an insect species is chosen first and the development of rearing practices follows. The goal is to optimise production to maximise the yield of that particular species in that facility. In contrast, the habitat-centric approach first chooses a habitat, either natural or artificial, then develops harvesting or rearing protocols within that habitat. The goal of this approach is to maximise the yield derived from that habitat and may include more than one species of insect. The habitat-centric approach eliminates potential threats from invasive species, and can repurpose local food and agricultural waste into protein derived from local insect species. This approach can increase food security by increasing the diversity of insects that are mass-produced. The species-centric and habitat-centric approaches address different issues and offer advantages in different situations. Further development of the edible insect industry will likely use a combination of both approaches.

Determination of the calorimetric potential of yellow mealworms in a mass rearing context

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During the last decade, insects have received increasing interest as sources of protein for animal feed and human food. Yellow mealworm (*Tenebrio molitor*), is considered one of the best options because is rich in protein, easy to breed and feed. This species of insect is commonly reared in crates containing agricultural by-products such as wheat bran. In a mass rearing context, an insect farm can contain thousands of these crates with different insect life stages. At the moment, there is only little data available concerning the heat generation of yellow mealworms in rearing conditions, making it hard to scale livestock houses. In this context, the goal of this study was to measure the energy expenditure (EE) of insects at different life stages in optimal rearing conditions (temperature, hygrometry, insect density, water supply) during twenty four hours using calorimetric chambers. More exactly feed consumption, body mass, heat generation, O₂ consumption and CO₂ release were measured for 8 insect life stages (i.e. eggs, 5 mg larvae, 10 mg larvae, 20 mg larvae, 40 mg larvae, 80 mg larvae, pupas and adults). Results obtained illustrate for the first time the calorimetric profile for each insect life stage in mass rearing conditions. Indeed, the EE was quite low for eggs and pupas whereas it increased together with the larval stage (from 0.0011 to 0.016 kcal/individual) and even more for adults (0.024 kcal/individual). Thus, these observations suggest a good calorimetric potential for advanced larval stages and adults. Further studies will be realised on the complete life cycle of *Tenebrio molitor* and will include the impact of the photoperiod. Taken together, these data could provide a key knowledge for the scaling and sizing of new yellow mealworm farms.

Growth performance of *Tenebrio molitor* sexed and non-sexed

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Optimisation of mealworm production is necessary to make it more cost competitive with other more established protein sources. The aim of the present study was to investigate reproductive efficiency, production, feed efficiency, and survival rate in sexed and unsexed *Tenebrio molitor* with the hypothesis that sexing insects increases their average life span and larval production. For that, eighteen experimental batches were prepared, which were divided into 9 batches with sexed insects, each batch containing 10 *Tenebrio* (5 females and 5 males). The remaining 9 batches were of unsexed *Tenebrio* with 10 adult *Tenebrio* at random. The beetles were fed with 250 grams of wheat bran and brewer's yeast (10:1, 20.5% CP, 18.97 MJ GE/kg DM). using 3 adult/ cm². Larvae (per box) at 120 d were weighed, the number of larvae, their weight and the weight of the remaining substrate were evaluated. Reproductive efficiency, feeding efficiency and survival were subjected to analysis of variance (ANOVA) using the general linear model (PROC GLM) of SAS (2002), Tukey's test was used ($P < 0.05$). It was demonstrated that sexed *Tenebrio* had a higher number of larvae (986) than unsexed *Tenebrio* (758) ($P < 0.05$), but a lower number of pupae and adults ($P < 0.05$), with better feed efficiency (g feed/g larva produced) for the unsexed (7.2) compared to the sexed (11.8) ($P < 0.05$) treatments, with a higher adult survival ($P < 0.05$) for the sexed *Tenebrio* at 80 d. Consequently, we demonstrated that the sexing of *Tenebrio* increases the number of larvae produced at 120 d, but increases mortality and cannibalism among them, giving a lower larval yield at 120 d compared to the use of unsexed adults. It is recommended to separate larvae every 7 days to reduce the risk of cannibalism.

Effect of graded levels of *Imbrasia belina* meal on blood lipid profile, bone morphometric and mineral content of broiler

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The effect of increasing dietary levels of *Imbrasia belina* worm meal on blood lipid profile, bone morphometric and mineral content in Arbor acres broiler chicks were assessed. A total of 360-day old chicks were randomly allocated to 4 dietary treatments, with each treatment group replicated 6 times (n=15/replicate). The inclusion levels of the *I. belina* worm meal in the diets were 0% (Treatment 1 = control), 4% (Treatment 2), 8% (Treatment 3) and 12% (Treatment 4). A 3-phase feeding program of starter (0-14 d), grower (15-28 d), and finisher (29-35 d) was employed for the study. Total cholesterol (TC), triglycerides (TGL), high density lipoprotein (HDL), low density lipoprotein (LDL), bone morphometrics and mineral content were recorded at the end of study. There was no significant difference of total cholesterol (CHOL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) concentration in all the treatments. However, significant concentrations were observed in triglycerides (TGL) and T3 showed highest levels compared to other treatments, at 35 days of age. The dietary inclusion levels of *I. belina* had no effect on femur weight, width, relative bone density, and bone breaking strength. However, the significant difference was observed on femur length, robusticity index and ash percentage. Also, broiler chickens fed T1 (6.62 g/100 g, 15.23 g/100 g dry matter) had the highest femur Ca and P content. Femur Z content of broilers fed T4 (24.08 mg/100 g dry matter) was highest. The correlation analyses showed significant moderate relationships between ash and femur weight, but however significant positive relationships with calcium, magnesium, manganese and zinc. More so, the correlation between femur strength and calcium and phosphorous was significant positive with a higher magnitude. The result of this study suggests that *I. belina* meal up to 12% can be effectively utilised in broiler diets without any adverse effect on and bone mineral properties

Influence of self-selection of feeding substrates by *T. molitor* larvae on nutrient intake and growth rate

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The yellow mealworm (*Tenebrio molitor*) is an edible insect and a promising candidate for industrial-scale breeding and production. To ensure balanced feeding and achieve favourable larval growth, it is necessary to regulate insect feeding and adapt feed composition to larval age. In our previous studies, we found that *T. molitor* larvae prefer certain feeds depending on larval stages. Nutritional self-selection was used to find out the optimal intake ratios of macro-nutrients for *T. molitor* larvae. The current experiments represent a new advance in the study of independent food selection by *T. molitor* larvae. Self-selection experiments consisted of 4 treatments of 8 ingredients, from a total of 26 different feeding substrates, with groups of 100 larvae. Six-, eight-, and ten-week-old larvae were reared for two weeks under optimal climatic conditions (27 °C, 75% rh) in a multiple-choice system, in which a free choice of available food was given and radially allocated in a round sieve. Larval weight gain, ingredient consumption and frass production were recorded to calculate efficiency of conversion of ingested food and food assimilation. It could be shown that the macronutrient requirement depends on the age of the larvae. Biomass increase was significantly influenced by intake of feeding substrate and carbohydrate content. Substrates with a high carbohydrate content were consumed most frequently, followed by protein content. Wheat bran, broad bean hulls, potato flakes, oat bran and flakes, rice flour and rice protein, corn husk and variegated thistle flour were identified as suitable for *T. molitor* larvae diets based on their relative consumption. These results indicate that feeding composition is critical for larval growth. It was also shown that larvae can self-select which macronutrients are needed at a certain time of development, so that targeted feeding during larval development can be implemented by a self-selection system. Based on this information it is also possible to evaluate diet formulations especially for *T. molitor* larvae in the future.

Optimal dietary protein to carbohydrate ratio for black soldier fly larvae

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To efficiently produce black soldier fly (*Hermetia illucens*, BSF) larvae for food and feed, larvae nutrient requirements need to be investigated. This study investigated the optimal protein to carbohydrate ratio for BSF larvae rearing. Six artificial isolipidic and isoenergetic diets varying in crude protein to digestible carbohydrate ratio (P:C) were formulated (increasing from 1:1 to 1:9) and compared to a chicken feed control (1:3). At the start of the trial, 10,000 of 5-day old larvae were placed on 7 kg of the different diets in triplicates and initial larvae and substrate samples were taken. For 11 days, larvae growth was measured daily and at the end of the trial, larval harvest yield and substrate residue weight was determined, and final larvae and substrate samples were taken. Initial and final larvae and substrate samples were analysed for their proximate composition. Overall, a higher P:C ratio resulted in faster larval growth and higher substrate dry matter reduction. However, larvae fed the highest P:C ratios had a significantly lower survival rate. Subsequently, the highest final larval yield was observed for larvae fed 1:2 and 1:3 ratios. These results suggest that the optimal dietary P:C ratio for larval growth and survival is 1:2-1:3. The optimal P:C ratio provides information on the nutritional requirements for larval growth and can be used for feed formulation.

Phase-feeding with *Torula* yeast modulates the production cycle in black soldier fly (*Hermetia illucens*)

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Maintaining a constant supply of high quality eggs is a major challenge for black soldier fly breeders. Feed supplementation with inactivated yeasts is known to enhance the quality of breeders and hatchery performance. However, yeast supplementation during the entire feeding phase is expensive and thus, unlikely to be applied at an industrial scale. Yeast-supplementation during targeted phases (i.e. phase-feeding) may permit gains in productivity. In the present study, BSF larvae were fed *ad libitum* (0.05 g ind./day) in triplicate with an inactivated yeast-enriched diet (*Torula* sp., 10%, dry basis) during the neonate (0 to 5 days post hatching) or prepupal (10 to 17 days post-hatching) phases and compared to larvae fed with a control Gainesville diet (70% RH) during the entire feeding phase (17 days). The pupae were then placed in small cubic breeding cages (28.5 cm²; 40 g of pupae; ~220 ind./cage) to complete their cycle (56 days post hatching at 30 °C; 60% HR; 12L:12D). Performance during feeding (substrate pH and T°, individual growth and feed bioconversion parameters) and breeding (emergence of flies, egg production, sex-ratio F:M and mortality) phases were monitored daily. At day 17, *Torula* supplementation did not affect growth or bioconversion performance. However, when compared to the control, supplementation was found to accelerate and delay adult emergence and egg production during the neonate and prepupal phases, respectively. Sex-ratio (F:M) mortality was also found to be significantly affected (0.6±1.0, 1.1±0.1 and 1.2±0.6 for control, neonate and prepupal diets, respectively). Once the optimal levels and duration are determined, phase-feeding approaches in BSF could allow to modulate with greater precision rearing processes and contribute to the sustainability of the industry.

Yeast rich residuals from local beer, rum and kombucha production as supplements in yellow mealworm diet

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Insect production offers the potential to revalorise residual sources of microorganisms from the agrifood sector. Yeasts can be added as feed supplements in edible insect diets like mealworms for their rich content in proteins and vitamins. They also produce attractive volatile that promote feed consumption, support growth and feed conversion efficiency. For convenience, yeast supplements are often commercially available dry brewer's or baker's yeast, however residual yeasts are relevant sustainable alternatives. The aims of this research are to confirm the potential of residual beer yeasts and evaluate the effects of other fermentation residues on the survival and growth promotion of yellow mealworms. In a preliminary experiment, humid spent brewery yeasts from blond, kolsh and lager beers were incorporated as frozen cubes (12.5 ml water equivalent 2×/week) in a yellow mealworm diet (150 g wheat bran/ 25 g larvae ≤1 mm) at 5% of the standard production scale. Total and individual larval biomass were increased in the kolsh (+18.8 and 14%, respectively) and lager (+21.3 and 17.3%) treatments. Solid dry kolsh and lager yeasts residues were left unconsumed at the end of the 69 days growth period and complicated larvae harvesting. In a test at 50% production scale, the supplementation of yeast with vegetable pulp increased individual fresh mass by 27% ($P<0.001$). The second part of this research investigates the metabolic effect and operational feasibility of supplementation of a mealworm diet with local residual yeasts (from ales, lagers, and rum), vegetable pulp, and SCOBY. Different mix recipes were tested for suitable texture to ensure easy handling. The formulation of gel supplements is still to be investigated. 5 treatments (n=3) of residual mixes were supplemented bi-weekly (5% w.w.) to a wheat bran diet (6 g/g larvae) during the exponential growth stage of larvae aged from 4 to 8 weeks at 25±3 °C and 60±10% relative humidity. These results will facilitate the supplementation of insects' diets by the revalorisation of local commercial yeast residues from beer, rum, and kombucha production.

Value and increased performance of black soldier fly mass-rearing farms

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Humanity is facing problems such as unsustainable utilisation of resources and unstable food security. Timely change of our ways is required. The rise of the insect food and feed industry is coming at the right time offering less environmental impacts in exchange of good quality protein. Insects have the potential to help us reach 7 of the 17 Sustainable Development Goals of the United Nations set up to achieve by 2030, underlining the vast potential of the industry. *Hermetia illucens* known as black soldier fly is one of the main species yielding substantial success. It is globally approved for feed in poultry, aquaculture and pet food. The interest in *H. illucens* comes from its high adaptability to different conditions and its comparatively fast life cycle. Other important advantage is the larva high protein content, antibacterial and hypoallergenic properties. Insect mass-rearing producers are most commonly investing more in the research and development of their zootechnical organisation, enhanced environment, and colony maintenance. The main priority in the field often falls on tracking bioconversion key performance parameters. In addition to that, our team is focused on the black soldier fly itself and its genetic capacity. The black soldier fly genome has been sequenced, unravelling genomic size larger compared to other Dipteran species (1,102 Mb) and significant allele variability giving potential for development of marker-assisted selection. There is now increasing evidence that genetically diverse populations manifest greater fitness, production, and reproduction capacity. In the long term there is evidence that ignoring the genetic landscape of your population could lead to genetic purging. Even if data is still limited, research begins to prove that if genetic healthcare systems are not established, inbreeding coefficient and consanguineous mating will elevate affecting the overall performance of the colony. All this underlines the importance of genetic rescue strategies in a commercial BSF farms. In our work, we describe the available research on *Hermetia* genetics and have underlined the species-specific biological significance for introduction of genetic health care system into a mass-rearing facility to sustain high performance colony for high investment returns.

The inhibitory effect of tofu developed from *Tenebrio molitor* larvae on dexamethasone-induced muscle atrophy

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This study aimed to develop tofu from whole (M) or protein isolate (MPI) or protein hydrolysate (MPH) of mealworm (*Tenebrio molitor*) larvae, an edible insect, and examine its inhibitory effect of dexamethasone (DEXA)-induced muscle atrophy, which is an abnormal condition regarding loss of skeletal muscle mass and function. In addition, we investigated the synergistic effect of plant- and animal-based proteins and the advantages of bioactive peptides. MPI was extracted from M powder, and MPH was prepared under optimised conditions. The tofu was prepared by replacing soybean flour with M powder (SM), MPI (SMPI), and MPH (SMPH) at 1:1 ratio by weight, and their properties were compared with those of control tofu (S). In the mouse model, bodyweight change in DEXA-induced muscle atrophic mice on SM (DEXA-SM; -0.07 kg) and SMPH (DEXA-SMPH; -0.39 kg) diet showed no significant differences ($P>0.05$) with that in the control mice (+0.47 kg) on a normal diet with no DEXA administration (DMSO con). Compared to that in DMSO con (0.044 N/g body weight), grip strength was not significantly different in DEXA-SMPH (0.044 N/g body weight, $P>0.05$). Furthermore, DEXA-SM had the greatest cross-sectional area (1,378.33 μm^2) among all groups. The mRNA expression levels of muscle degradation-related genes such as myostatin, MuRF 1, and Atrogin-1 were inhibited in DEXA-SM and DEXA-SMPH, with no significant differences ($P>0.05$) in the expression levels between DEXA-SMPH and DMSO con. However, there was no significant difference in the mRNA expression levels of MyHC isoforms which are related to muscle synthesis in DEXA- and tofu-treated mice ($P>0.05$). Based on these results, skeletal muscle atrophy caused by protein degradation can be alleviated by SM and SMPH intakes. They are worth having the significant potential for inhibiting DEXA-induced muscle mass and strength loss.

Polygamy in *Hermetia illucens*

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Black soldier flies (BSF) are an emerging source of insect proteins around the world. One of the main challenges in rearing BSF at industrial scales, especially indoors, remains the efficient production of eggs. Research on BSF reproduction is mostly focused on population-level metrics, but little is known about the mating behaviour of individuals. Here, we investigated the mating behaviour and phenology of adult BSF by uniquely marking individuals in 1:1 sex ratio groups and monitoring their mating and laying event with automated cameras. Our results show that males and females can have multiple sexual partners and that females can mate and lay viable eggs twice. Our results suggest the potential interest of having a female-biased sex ratio in large scale productions, as males can copulate more than once and rearing space is limited.

Towards a better understanding and predictability of pupation in *Hermetia illucens*

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The black soldier fly (BSF) is a promising insect species for the bioconversion of food waste into high protein food. The waste bioconversion industry by BSF is only possible with the maintenance of a colony capable of regularly producing new larvae. Throughout the BSF lifecycle, pupation is one of the most challenging aspects of rearing, as it is the longest and most variable period of their life cycle. A synchronised emergence of adults benefits reproduction, therefore, being able to get a stable duration of pupation and a synchronised adult fly emergence is a major advantage for industrialised rearing operations. Here, we identified a series of factors that influence pupation duration and fly emergence synchrony. Body size, larvae density, larvae diet and rearing temperature all impact the duration of pupation. Meanwhile, emergence synchrony increased with the rearing temperature. Interestingly, adults emerged according to strict circadian rhythm, most of them emerging between 10:00 and 15:00. By controlling these different factors prior or during the pupation state of BSF, we can obtain a more predictable duration of pupation and a more synchronised emergence which benefits the black soldier fly breeding operations.

Agricultural entomophagy: insect rearing for human consumption and feed

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As the human population grows, it is very important to sustain rather than increase the levels of consumption. Scientists are struggling to find alternative ways to feed the ever-increasing population. Current sources of protein are not sustainable. Half of the world's habitable land is used for agriculture. More than three-quarters of this is used for livestock production. Insects require 10 to 50% less of the amount of water than other animals use for food and land per pound of protein. Insects utilise less energy, feed, land and water than livestock; therefore, contributing less to climate change and pollution. Developing and evaluating protein extract made from mealworms and crickets will aid food companies in their goal to add higher levels of insect protein to products without adding fat, calories or negatively affecting product quality. Our objectives are 1. Determine the optimum density and temperature for rearing edible insects, and 2. To study feed optimisation by adding cannabidiol (CBD) to the diet of the edible crickets, *Gryllodes sigillatus* (banded cricket). As the density of the crickets and temperature increase, the wellbeing of the insects significantly reduces. The optimum density seems to be 66/crickets per container (13.63 in. × 8.25 in. × 4.88 in.) at either 75 and 85 °F growth chambers. The preliminary result on the CBD diet revealed that insects fed with 500 mg CBD oil-treated showed increased and reduced mortality. Further research is underway on the level of CBD on cooked crickets and the optimisation of the diet.

Insect production systems/processing organic side streams & waste management

Effects of microplastics on manures processing by black soldier fly larvae

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Bioconversion by black soldier fly (BSF; *Hermetia illucens*) (Diptera, Stratiomyidae) larvae is a novel technology for manure (poultry, swine, cattle) treatment and valorisation in agriculture. However, some microparticles of plastics present in all ecosystems may have negative effects on BSF larvae production. Moreover, the efficiency of manure degradation may also be affected by microplastics (MPs). In this study, three types of bioplastics were tested in the experiment: (1) polylactic acid (PLA); (2) polybutylene succinate (PBS); (3) polybutylene adipate terephthalate (PBAT). These bioplastics were ground into small particles of size 250 µm using a Retsch cryomill. About 1% of MPs was mixed into substrate rearing (poultry and swine manure). Each of the three experimental experiments was carried out in three independent replications in plastic boxes. The experiment was conducted in a laboratory room under controlled conditions (27±2 °C and RH 60±5%). Each container contained 170- 3 day old larvae and 300 g of substrates (1/3 poultry manure and 2/3 swine manure). The larval density was one larva by 1 cm² (based on literature data and our preliminary trials). Some physiological parameters of BSF were measured until pupation. The weight of BSF was measured every 2-4 days (growth rate). The development time to first prepupation was recorded upon observation of the first prepupa within a given container rates and adult emergence was determined at the end of the experiment. Survivorship was calculated by dividing the total weight of all living larvae from each replicate by the average final larval weight. The prepupal weight was measured by selecting 10 of the largest prepupae from each replicate. Therefore, the efficient biodegradation of each substrate by BSF (feed conversion ratio and feed efficiency) were controlled for each replicate. Finally, 30 larvae were digested by protocol published in Al-Azzawi *et al.* and were analysed by FTIR for each experimental treatment.

Research and application of organic waste conversion by black soldier fly larvae and microbes

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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 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 play important roles in BSF larvae nutrition and resistance against pathogens. 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 reduced 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 nematicidal microbes to produce functional organic fertilisers and have good efficiency against root rot nematode in field. The technology combined BSF and microbes could reach low emission and high-value utilisation of organic wastes.

Biowaste conversion and growth of BSF larvae on substrates containing similar crude protein content

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A series of experiments were performed to study black soldier fly larvae (*Hermetia illucens*) growth performance and safety on different biowaste sources. In this experiment five biowaste sources were used, solely or in combinations: fast food (FF), mushroom feet (stems) (MF), poultry meal (PM), slaughter waste (SW), and pig manure solids (PS). The following six diets were formulated based on 30% dry matter and 20% crude protein: FF, FF-MF-PM, FF-MF-SW, FF-PS, PS-MF-PM, and PS-MF-SW. Per kilogram (wet) diet, 1,850 BSF larvae (8 days of age, 12.0 mg fresh weight) were incubated in 18 plastic containers (75×47×15 cm) (each diet in triplicate) in a climate chamber under dark conditions and were harvested at 15 days of age. Diets affected the growth performance of larvae, waste reduction, efficiency of conversion, and larval biomass ($P < 0.05$). Larval growth rate in mg/d was highest in FF (18.0^a), followed by FF-PS (14.9^b), FF-MF-SW (12.3^c), FF-MF-PM (10.0^d), PS-MF-PM (7.8^e), and PS-MF-SW (7.3^e). Waste reduction index was highest in FF-MF-SW (7.1^a) followed by FF-MF-PM (6.4^{ab}), FF-PS (6.2^b), FF (5.8^b), PS-MF-SW (4.5^c), and PS-MF-PM (4.3^c). Efficiency of conversion of ingested substrate was highest in FF-PS (0.49^a) followed by FF-MF-SW (0.41^{ab}), FF-MF-PM (0.38^{bc}), PS-MF-PM (0.31^{cd}), PS-MF-SW (0.28^d), and FF (0.23^d). Total larval biomass (g DM) at harvest was highest in FF-PS (777^a) and FF-MF-SW (734^a), followed by FF-MF-PM (627^b), FF (390^c), PS-MF-PM (349^c), and PS-MF-SW (345^c). The results implicate that despite similar dry matter and crude protein content in the different diets, biowaste conversion and growth of BSF larvae vary to a great extent. The advantage of fast food in larval growth rate was counterbalanced by the low survival on this diet, most probably due to the high crude fat content which has still to be confirmed by analyses of chemical data. Mixing fast food with other biowaste sources improved survival, production output and waste conversion.

Oyster mushroom-fermented corn stover in feed impacts cricket growth performance and micronutrient bioavailability

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Due to rapid growth and nutrient density, farmed insects could help combat public health problems such as protein energy malnutrition and anaemia, but reliable low-cost feeds are needed. Here, we explore the potential to modify corn crop residue with edible mushroom mycelium to generate a feed adjunct for edible *Gryllus bimaculatus* grown in contexts such as rural Zambia. Like insect agriculture, mushroom farming is versatile; it can yield nutritious food while increasing system circularity by using lignocellulosic residues from row crops as inputs. The oyster mushroom, *Pleurotus ostreatus*, is an edible basidiomycete widely cultivated on corn stover (*Zea mays*). Mushroom harvest results in abundant 'spent' substrate, which we investigated as a low-cost cricket feed adjunct. We measured cricket growth performance and micronutrient content for six fermented feed treatments and an unfermented control, testing impacts of fungal fermentation duration and induced mushroom formation. Pasteurised corn stover was inoculated with *P. ostreatus* mycelium and fermented for 0, 2, 3, 4, or 8 weeks, and some 4 and 8-week substrates were induced to produce mushrooms. Dried fermented stover (40%) was added to a 1:1 corn/soy grain mix and fed to crickets *ad libitum* for 44 days. Cricket mass, mortality, and maturation were measured weekly. The unfermented control group showed higher survivorship compared to several fermented diets. Total mass yield was $16.5 \pm 10.7\%$ higher in crickets fed unfermented control diet than the combined mean for all fermented diets. Little variation in cricket iron content was observed via ICP-spectrometry across feeds, averaging $24.6 \pm 1.0 \mu\text{g/g}$. To determine iron bioavailability, we conducted *in vitro* Caco-2 human colon epithelial cell absorption assays, showing that iron in crickets fed fruiting-induced stover was more bioavailable than in unfruited treatment groups. Despite more bioavailable iron in crickets reared on post-fruiting substrates, we conclude that *Pleurotus*-fermented stover is an unsuitable feed ingredient for *G. bimaculatus* due to high mortality and poor yield.

Autonomous modular waste management infrastructure using black soldier flies

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Waste management is evolving. To build a circular economy we need to redesign the waste management system from the ground up. Globally food waste is directly responsible for 8% of carbon emissions. One third of all food produced is lost or wasted (1.3 billion tonnes), costing the global economy c.\$940 billion each year. The biggest contributors of food organic wastes come from households and businesses, often at low volumes and unpredictable composition. Inefficient logistical solutions for the transportation of this waste and nearby infrastructure exacerbates the issue further and has seen little change over time. We require Infrastructure that is agile enough to manage different organic waste types and their associated logistics requirements. Insect bioconversion is a growing trend but still proves to be inaccessible and costly for many towns and cities. Goterra has developed the methodology to unlock technology driven solutions to enable access to insect bioconversion whilst redesigning waste management solutions using black soldier flies. Goterra explores solutions to conveying, accepting and processing waste and ensuring the insects will consume it from a start-ups perspective. An autonomous, modular solution has been developed to bridge the logistical gap by enabling the separation and bioconversion of packaged organic waste onsite through BSF's. The bioconversion from the BSF's also produce highly sustainable protein and soil enhancer onsite. This solution involves a modular infrastructure for biological services (MIBS), where each unit can process up to 5 tonnes or packaged waste a day. It has been proven that a MIBS stack has a practical time to market starting at 6 months at a competitive rate and little to no disruption to the site's current waste removal process. This method can be easily adopted by any city or town that is both environmentally and financially sustainable. The research explores the challenges faced using rapid prototyping in parallel with design and delivery to the client. Continuous learning and learner experience design onsite help support applied research and experimental development carried out by Goterra. This relationship enables development of a robust solution when dealing with varying and contaminated organic wastes based on food and packaging trends.

Effect of food attractants on the oviposition rate of the black soldier fly (*Hermetia illucens*)

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The black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), is a cosmopolitan fly. Its feeding behaviour, high productivity and the high value of its larvae in macronutrients (mainly lipids and proteins) makes this species one of the promising candidates for large scale insect farming and organic waste management. The reproductive rate, female oviposition preference and oviposition attractants of *H. illucens* are understudied. A better knowledge of the attractants involved in oviposition site choice would help maximising black soldier fly reproductive success. The current study aims to compare the efficacy of four food attractants on the oviposition activity of *H. illucens* females. The trial was conducted in a cage (11×1.25×0.9 m) with a volume of 12.38 m³ and containing 92,900 adults (7,500 adults/m³). The cages were placed in a rearing room where the conditions were: T=28±1 °C, RH=70±5%, photoperiod of 16 h light:08 h dark and light intensity = 8,140 Lux). The tested feeds included fresh cattle manure, a mixture of tropical fruits (pineapple, banana, papaya and mango), a rabbit feed, and fish waste (sardine, sardinella, and anchovy waste). The performance of each feed attractant was evaluated in terms of the mass of eggs laid on the laying medium. This study showed that fruit mixture is the most efficient attractant, followed by fresh cattle manure and fish waste while the rabbit feed was the least efficient. The results of this study can be used to optimise the reproduction of the black soldier fly.

Life-history traits of the black soldier fly reared on differing diets: from larval development to egg production

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The black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), has been proven as a method for managing organic side-streams (e.g. food waste, manure) as a means to produce a high-quality feed while protecting the environment (e.g. reduction in noxious volatile organic compounds). In this study, BSF larvae were fed different food waste products (e.g. oranges, carrots, brewers grain) that are common in Texas, USA to determine the impact on life-history traits from larval development to the reproductive output. We determined that some life-history traits such as total larval biomass at harvest did not differ based on diet, but other life history traits such as number of larvae at harvest did. These data indicate that while BSF larvae are more efficient at converting some food waste products than others, valuable production products (larvae, adults, and fertile eggs) can be produced on the diets utilised in this study.

Zero waste protein factories: leveraging biology to empower the industry for waste-to-protein conversion on-site

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A fifth of all the food that is produced in Europe becomes waste, equating to 88 million tonnes of food being lost on a yearly basis, with associated costs estimated at 143 billion euros. About 30% of food waste is being produced during processing or manufacturing. A typical food manufacturer company can easily produce 5,000 to 10,000 tons of organic by-products every year. Many food companies have made the reduction of food waste one of their main sustainability goals for the near future. Insect farming could significantly contribute to the reduction of food waste and a circular economy, reintroducing nutrients from food waste directly into the feed chain. Also, the entrepreneurial appetite for integrating revenue-generating zero waste solutions such as insect farming is growing. However, implementation of insect solutions for waste upcycling on an industrial scale has been facing several challenges. Besides cost and sustainability concerns with transport and logistics, the biological and economic feasibility of insect farming on various by-products remains a great unknown for industrial food processors. This keynote demonstrates that, by diligent assessment of biological feasibility, 'zero waste protein factories' utilising black soldier fly larvae (*Hermetia illucens*) for 'on-site waste-to-protein conversion' could be economically feasible, already at a small industrial scale. The assessment factors include the biological assessment of feed substrates and feed formulas, scale of the factories, as well overall economic feasibility. It highlights the potential for industry players to not only save costs on disposal of their by-products but turn it into revenues while saving emissions as compared to composting or biogas digestion.

Influence of (micro)plastics on black soldier fly larvae (*Hermetia illucens*) growth performance and bioconversion

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Packaging plastics are currently used globally in a myriad of food applications. Consequently, they are found in energy-rich biological waste derived from the food industry, supermarkets, restaurants, and catering. Black soldier fly (BSF) larvae (*Hermetia illucens*) are voracious feeders that can be reared on a wide range of biological materials and turn such low-value waste into valuable biomass. Nevertheless, the presence of packaging plastics might affect their growth, bioconversion, and chemical safety. In this study, it was investigated whether: (1) the contamination of substrates with plastics affects larval growth and bioconversion; and (2) BSF larvae are able to clean contaminated plastics adequately. To investigate these aims, BSF larvae were reared on substrates (667 and 1000 mg/larva) containing different amounts ($w=1-5\%$, on *as is* basis) and sizes (macro-, meso- and microplastics having a width of 5 cm, 5 mm, and 143 μm respectively) of polyvinyl chloride (PVC) packaging foil. During this experiment, the larval growth performance and the bioconversion parameters (food conversion ratio, waste reduction ratio, and efficiency of conversion of digested and ingested food) were determined. Afterwards, the remaining plastics were quantified and analysed upon potential polymer degradation and oxidation induced by the BSF larvae. The BSF larvae grew well on all substrate types included in this study, and a mean maximal larval weight of 194.5 mg was obtained. It was also found that the presence of PVC did not affect the survival rate and bioconversion parameters, while the BSF larvae were able to decrease the amount of substrate present on the plastics considerably. In addition, it appeared that the impact of the larvae on the physicochemical properties of the PVC pieces and particles was negligible.

INSECT4CITY: benefits and risks of insect-based bioconversion for recycling bio-waste from urban and peri-urban areas

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In urban and peri-urban areas, where waste management is more than ever a major issue, insect farming may be an ecological way to recycle bio-waste in a circular bioeconomy approach. Some insect species such as the black soldier fly (*Hermetia illucens*) can grow on many types of organic substrates and can convert them into valuable by-products. There may be a wide variety of insect bioconversion products since larvae are a natural source of micronutrients, amino acids and antimicrobial compounds (chitin, lauric acid or antimicrobial peptides). They are also rich in proteins which can be used for animal feed, in fats used for the production of biofuel, in chitin used in various industries, and they produce a type of manure called frass that can be used as biofertiliser. However, besides its ecological and nutritional benefits, risks of insect-based bioconversion should be identified and monitored. Indeed, some chemical contaminants may be found in insect farming environment, in their feeding substrates or can be produced during processing methods. Moreover, microbiological contaminants may be found in the insect farming chain. Therefore, it is necessary to assess the impact of these contaminants on insect production but also to determine their fate in the insect (bioaccumulation or biodegradation). It is also important to assess the environmental, social and economic impact of insect-based bioconversion and to determine the consumer acceptability of these new processes and products. It is around this overall benefit-risk assessment that the project INSECT4CITY is focused, bringing together 8 research divisions of INRAE, the French National Research Institute for Agriculture, Food and the Environment. It will address, upstream of the development of this sector with very high potential, all the questions that the general public, the legislator as well as the producers of insects can ask themselves about this means of recycling bio-waste.

Brewing by-products as rearing substrates for black soldier fly: effects on larval growth and conversion efficiency

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Brewing by-products are recognised as suitable rearing substrates for black soldier fly (BSF), but the potential of using brewer's spent grains (BSG) and brewer's spent yeast (BSY) at different dietary ratios has rarely been investigated. This study evaluated the effects of BSG-BSY-based diets on BSF larval growth, survival, and conversion efficiency. A total of 3,000 6-day-old BSF larvae were allotted to 5 dietary treatments (6 replicate boxes/diet, 100 larvae/box, 0.9 g diet/larva): (1) BSY2.5 (97.5% of BSG + 2.5% of BSY); (2) BSY5 (95% of BSG + 5% of BSY); (3) BSY7.5 (92.5% of BSG + 7.5% of BSY); (4) BSY10 (90% of BSG + 10% of BSY); and (5) control (GA, Gainesville diet). Larvae were individually weighed (at least 30/replicate) every 4 days (10 and 14 days of age) and at the end of their growth (5% of prepupae), and their survival was calculated. Bioconversion efficiency corrected for residue (BER), reduction rate (RR), and waste reduction index (WRI) were also calculated. Data were analysed by means of GLMM (IBM SPSS software, $P < 0.05$). The BSY10 larvae overall showed the highest weight among the BSG-BSY-based diets, but lower than GA ($P < 0.05$). At 10 days of age, BSY7.5 and BSY10 larvae displayed higher weight than BSY2.5 and BSY5 ($P < 0.05$), with BSY10 and GA being similar ($P > 0.05$). The 14-day-old larvae were also heavier when fed BSY7.5 and BSY10 diets than the other BSG-BSY-based diets ($P < 0.01$), but lighter than GA ($P > 0.05$). At the end of growth, the BSY10 larvae showed the highest weight among the BSG-BSY-based diets ($P < 0.05$), being analogous to GA ($P > 0.05$). Survival rates were similar among the diets ($P > 0.05$). All the BSG-BSY-based diets were characterised by higher BER than GA, with BSY7.5 and BSY10 larvae also displaying a better BER than BSY2.5 and BSY5 ($P < 0.01$). Similar RR and WRI were observed among the BSG-BSY-based diets ($P > 0.05$), but lower than GA ($P < 0.05$). In conclusion, including 7.5 and 10% of BSY in BSG-based diets led to the best results in terms of BSF larval growth and conversion efficiency. The study was funded by Fondazione Cariplo (CELLOW-FeeP).

Agricultural systems of the future – sustainability through connecting insects, plants, and fish production systems

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In light of the current developments in climate change, growth of world population, unprecedented urbanisation, and increasing diet-related diseases, there is an urgent need for a transformation in the food and agricultural systems. The aim here is to present and discuss the approach of CUBES Circle (Closed UrBan Modular Energy- and Resource-Efficient Agricultural Systems) which mimics an agro-ecosystem and connects production units such as of insects, plants and fish. This allows closed fluxes of resources while producing healthy food under controlled conditions. The residues of the production in the higher trophic level, (e.g. fish) are used as substrates for the production of food from a lower trophic level (e.g. plants and insects). This means that resources (H₂O, CO₂, micro and macro nutrients) as well as the resultant energy and exergy will be recycled inter-connecting the trophic levels in the system. Since circularity and the principle of zero-waste are the most important features of this system, using a generalist saprophage such as the black soldier fly in the CUBES Circle offers the desirable advantages in filling the connection gap between plant and fish production.

Effect on environmental impact of circular feed inclusion in large-scale production of *Alphitobius diaperinus*

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Larvae of the *Alphitobius diaperinus* (commonly known as buffalo mealworms) are reared on a large scale in the production facility of Ynsect (formerly Protifarm), in the Netherlands. These larvae are currently used for both feed (petfood and aquaculture) and food applications. Generally, insects are regarded as efficient converters of biomass into valuable components like protein, but for optimal growth buffalo mealworms require sufficient nutrients in a balanced diet. As for other livestock species, also in insect rearing a large share of the environmental impacts are associated to the feed used to grow the larva. The use of circular ingredients in larval diets might reduce the environmental impact of larval production, but it is unclear if the potential environmental benefits could be counteracted by a negative effect on larvae growth. In our study we investigated the effect of circular diets on growth, performance and environmental impact of *A. diaperinus*. For that, growth trials were performed in a Production Simulation setting with large-scale commercial conditions and the environmental impacts were estimated using data from a large-scale production facility using a life cycle approach. Circular diets resulted in a 12 to 18% reduction of larval yield. Despite the lower yields, the environmental impact per ton of larvae was reduced by 35%, more than 50% and up to 33% for climate change, water use and land use, respectively, compared to the standard diet currently used at commercial scale. Other environmental impacts such as resource use (fossil) were increased with the circular diets. Although the results are promising, other factors such as the cost-price of circular feeds and changes in the rearing system, need to be evaluated for their inclusion at a large-scale commercial setting.

Evaluation of waste-based substrates for a black soldier fly farm in Guinea-Bissau

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Access to high quality feed is challenging for poultry farmers in developing countries. In cooperation with the university of Turin, a community of poultry farmers in Guinea-Bissau started a pilot black soldier fly farm to evaluate the feasibility of producing their own poultry feed. The aim of this study is to assess the performance of two diets, formulated using locally-sourced waste. During the farm set-up, 5 waste streams were identified: dried cashew peels (DCP), chicken manure (CM), spent brewer's grains (SBG), rice husk (RH), and broken hen eggs (BE). The first diet (AF1), was composed by DCP (17.68%), CM (8.58%), RH (8.58%), BE (1.98%) and water (63.73%), while the second diet (AF2) contained DCP (17.68%), SBG (17.68%), BE (1.98%) and water (63.73%). Gainesville diet (GA) was used as control. A total of 1,800 6-day-old larvae were weighed in groups of 5 larvae and divided in 18 boxes (6 replicates/diet, 100 larvae/replica) containing 1.8 g diet/larva. Larvae were weighed individually every 4 days until reaching 40% prepupae. Survival, reduction rate (RR), waste reduction index (WRI), bioconversion efficiency corrected for residue (BER), and time of development from larvae to prepupae (L-P time) were evaluated. Data were analysed by means of GLMM (IBM SPSS software, $P < 0.05$). Larvae reared on AF2 showed greater weights than AF1 at 10 days (T1) and at the end of the larval stage (END) ($P < 0.05$). Larvae reared on GA showed greater weights than both the experimental diets at T1 and END ($P < 0.001$). Survival was similar in GA and AF2 ($P > 0.05$), but greater in both the diets than AF1 ($P < 0.05$). The RR was higher in GA than AF1 and AF2 ($P > 0.05$), while AF1 and AF2 did not differ ($P > 0.05$). The WRI showed comparable results, with GA higher than AF2, and AF2 higher than AF1 ($P < 0.05$). The BER was lower in AF1 than in GA ($P < 0.05$), but AF2 was similar to both the diets ($P > 0.05$). The L-P was faster in AF2, followed by GA and AF1 ($P < 0.05$). Prepupa weight was the greatest in GA, followed by AF2 and AF1 ($P < 0.05$). Concluding, AF2 yielded heavier larvae and prepupae, as well as a faster L-P, better survival and higher BER than AF1, proving to be the most suitable diet. Study was founded by the Fondazione Cariplo project CELLOW-FEEP.

Conversion of canteen waste along one year operated by black soldier fly larvae for evaluating heavy metal accumulation

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In the world, there are about 244 million t/year of waste from canteens and restaurant, while 4.7 million t/year only at the Italian level. Canteen waste (CW) could be used for feeding larvae of insect to obtain new marketable product (e.g. feed and frass). Black soldier fly larvae (BSFL) are voracious and polyphagous and CW is a complete food for them. Furthermore, BSFL are capable to bioaccumulate some heavy metals thus allowing to obtain safe organic compost, but it requires to evaluate the bioaccumulation for feed purpose. The CW was sampled once a month and the sampling was carried out for a year from a local canteen, located in the Northern part of Italy. The feeding was conducted in quadruplicate in plastic containers (12 l) placed in a rack inside a climatic chamber with 27 °C and 70% RH. In each box were allocated 2,000 g of CW and 2,000 larvae of 5 days-old. Trial ended at 7 days and growth performances was evaluated. Initial diet, neonates, BSFL and residue were sampled and oven-dried. Each sample was treated with acid digestion and with microwave. Elemental analyses were performed with a spectrometer mass in triplicate for evaluating 27 elements. The aim of this research was to evaluate the CW as BSFL rearing substrate investigating heavy metal accumulation and growth performances in BSFL. The results reported an average of final larval biomass along the year of 227.0 ± 114.95 g and a frass of 378.0 ± 140.59 g. The percentage of dry matter for initial diet was $26.1 \pm 10.31\%$, for final larval biomass was $35.3 \pm 6.55\%$ and for the residue of $57.9 \pm 19.24\%$. The ingestion of feed for BSFL was 298.1 ± 168.10 g during the trial. As, Pb, Hg, and Cd are regulated at European level for feed between the heavy metals analysed in this study. As, Pb, Hg, and Cd were under the maximum levels in CW for initial diet, neonates, residues and BSFL. The use of CW seems available for BSFL rearing. The results showed good growth performances and heavy metal values under the maximum limits indicated by the EU legislation. The hazard connected to the heavy metal accumulation operated by BSFL did not be recorded by our results.

Microbiological impact of heat treatment on supermarket waste as substrate for black soldier fly (*Hermetia illucens*)

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About 13% of the global food waste originates from supermarkets. This supermarket waste can be reused as valuable substrate for rearing black soldier fly larvae (BSFL) and be converted into high value biomass. An important point of attention is the possible presence of food pathogens, since substrates for insects should meet legal regulations and microbiological criteria. Hence, there is a need for treatments that reduce harmful organisms, such as a heat treatment. As part of the FACCE SURPLUS UpWaste project, supermarket waste was simulated by standardised mixing of supermarket foods (without meat or fish), followed by inoculation with *Salmonella* Typhimurium and *Staphylococcus aureus*. Different temperature-time combinations were applied on this supermarket waste, taking into account the coldest point of the sample. Microbial counts were determined before and after the heat treatment. Afterwards, heat treated supermarket waste was stored for two days at room temperature as well as in the refrigerator, and microbial counts were determined after storage. In addition, the selected temperature-time combination was applied on supermarket waste containing meat and fish and on non-inoculated supermarket waste, stored at room temperature for two days before the heat treatment. While results showed that a heat treatment of 50 °C on supermarket waste was insufficient to result in a microbiologically safe substrate, a treatment of 60 °C for 10 minutes succeeded to reduce the Enterobacteriaceae and *S. aureus* to below the detection limit of 1 log cfu/g. Results also showed *Salmonella* to be absent in 25 g of supermarket waste. Storage of the heat treated supermarket waste, both at room temperature and in cooled conditions, did not increase the Enterobacteriaceae and *S. aureus* counts and still resulted in absence of *Salmonella* in 25 g. Experiments with supermarket waste containing meat and fish and stored supermarket waste (which are currently being performed) will reveal whether the selected combination of 60 °C for 10 minutes will also be sufficient to result in a microbiologically safe substrate for BSFL.

Evaluating 15 different by-products from greenhouse production as a wet feed for the yellow mealworm

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Entomoponics has been defined as a production system that combines insect rearing and hydroponic greenhouse production in the same environment. It is considered a way of increasing profitability by making more efficient use of space and energy and reducing processing costs for organic waste. In an entomoponics setup we valorise the unused, climate controlled space underneath the crop supporting gutters, which are suspended above the ground for ergonomic reasons. Feasibility of a combined mealworm (*Tenebrio molitor*) and cucumber cultivation has recently been proven. Mealworms are generally fed with dry wheat bran supplemented with a moisture containing food source. This moisture source could be by-products originating from the vegetable production which would enhance the circularity of this cultivation technique. In this study 15 different plant-based by-products were evaluated as a moisture source for mealworm cultivation in a tomato greenhouse. Residues from the following crops: cucumber (fruits and foliage), tomato (fruits from two varieties), zucchini (fruits), bell pepper (red, yellow and green fruits, and foliage), eggplant (fruits and foliage), strawberry (fruits and foliage) and lettuce (foliage and roots), were supplemented *ad libitum* and results were compared with the reference agar-agar. Mealworms were reared in 60 by 40 crates in four replicates per treatment. The effect of the food source on the larvae growth rate and total insect production was determined. In addition, the nutritional value of the mealworms was determined (for all treatments) as well as the potential presence of pesticides (for four of the treatments). Pesticide exposure may have occurred both through direct contact when pesticides were applied to the crops above or indirectly through their feed.

Ammonia pretreatment of agri-food wastes to enhance black soldier fly larvae bioprocessing performance

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Black soldier fly larvae (BSFL) bioconversion is a promising bioprocessing technology. However, one major challenge in BSFL waste processing, is that agri-food wastes (e.g. animal manure, agricultural crops and residues) tend to be low in macronutrients and high in lignocellulosic fibre (e.g. cellulose, hemicellulose and lignin), resulting in longer larvae development times and lower larval mass compared to when reared on high-value substrates (e.g. food waste). This study aimed to increase BSFL process performance on four fibre-rich substrates by ammonia substrate pretreatment: brewers spent grain, grass clippings, oat drink-by-product and cow manure. We first conducted experiments to identify optimal aqueous ammonia dose (1 vs 5%, 25% conc.) and pretreatment time (3 vs 7 days) by comparing its effect on the lignocellulosic substrate composition (neutral, acid and lignin detergent fibre) compared to an untreated control. Once we determined the optimal pretreatment condition, we completed controlled feeding experiments with four replicates, using ammonia pretreated and untreated substrates in a climate chamber (9 days, 2.5 larvae/cm², 28 °C and 44-70% relative humidity). Results demonstrated that for most substrates a dose of 5% achieved improved lignocellulosic degradation, decreasing neutral detergent fibre by 6 to 22%, when compared to the untreated control. A pretreatment time of 3 days performed best for all substrates, except grass clippings, which demonstrated better results at 7 days. Based on our results, aqueous ammonia can result in fibre degradation of BSFL substrates and influence larvae performance. Although not fully evident at this stage of the experiments, ammonia pretreatment of such fibrous wastes shows mixed results of larvae performance depending on the substrates in question. This research works to increase performance of abundant fibrous low-value waste streams for recycling within the food system.

Black soldier fly larvae transform blends of dairy manure and soybean curd residue

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The growing number of dairy farms results in significant manure production, which, when it's not properly disposed of, creates a hazard to the environment. Dairy manure has a high fibre content, which makes it difficult for the larvae of the black soldier fly (BSF) to convert it into biomass. Therefore, the purpose of this study was to determine the effect of co-digestion of dairy manure and another environmentally hazardous waste source: soybean curd residue. The waste sources were given to BSF larvae at various mixing ratios, based on the waste source. It was discovered that mixing dairy manure and soybean curd residue in a 2:3 ratio produced the best results in terms of development time (21 days), survival rate (98.4%), waste mass reduction (75.4% wet and 56.6% dry waste mass), bioconversion (11.6% on a wet and 14.6% dry mass base), feed conversion ratio (6.4 wet and 4.0 dry), nutrient utilisation (nitrogen 62.1%; phosphorus 52.9%; carbon 66.4%), and fibre conversion (cellulose, 64.9%; hemicellulose, 63.7%; lignin, 36.9%). Based on the findings of scanning electron microscopy, we may infer that black soldier fly larval activity did lead to structural alterations in cellulose, hemicellulose, and lignin. We hypothesise that such changes in surface and texture are beneficial to the associated bacteria and, as a result, contribute to waste reduction as well as larval development. Our findings indicate that *Hermetia illucens*' co-conversion of dairy manure and soybean residues might play a significant role in the management of dairy manure.

Valorising potato waste using black soldier fly, *Hermetia illucens*

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Disposal of cull potatoes often presents a challenge to potato growers. If left unattended, piles of cull tubers serve as a reservoir of several important plant diseases. Their biological conversion into protein- and fat-rich biomass using larvae of black soldier flies, *Hermetia illucens*, may offer a solution to this problem. We conducted a series of experiments testing the suitability of cull potatoes for larval growth and development. Live tubers, whether intact or cut, were not suitable for the larvae. At the same time, shredded, frozen and then thawed, and cooked potatoes were a good substrate for growing larvae. Biomass accumulation increased with the increased amounts of potatoes given to larvae in a dose-dependent manner. Nutrient profile of harvested larvae was comparable to that reported in literature. Larvae fed on raw potatoes had significantly more ash compared to the larvae fed on cooked potatoes. There were no differences in other nutrients and in the caloric values. Potato vines did not support larval growth by themselves but did not impede it when mixed with shredded tubers. Black soldier flies may be successfully employed when other ways to utilise unmarketable tubers are not available or practical for potato farmers.

Mealworm productivity and nutritional value upon growth on varying grain residues

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To evaluate the potential of mealworms to upcycle milling residues two experiments were conducted to: (1) evaluate the substitution potential of 8 types of grain residues compared to a wheat bran diet (control); and (2) develop a recipe based on residual grain availabilities and larval biomass/productivity results. A total of 560 g of dry grains were placed in triplicate 6 l containers, with a substitution ratio varying between 5, 10, 20, 60 and 100% of cereals. 25 g of 46 days old larvae were placed in experimental bins at 25±2 °C and 65% humidity. Over 52 days, 21.0±3.5 g of carrot pulp (71.8%±3% moisture) were provided twice a week. Individual larval biomass, mortality rate, and time to metamorphosis were monitored. Results were analysed using a two factor repeated times ANOVA (grain × substitution rate). Based on total fresh larval biomass, 4 grains were equal or better than the control below 60% substitution (milling dust, soy meal, buckwheat and spent brewery grains). Other grains decreased biomass at increasing inclusion ratios (corn, oat and barley screenings, bakery residues and canola). In experiment b, 6 diets containing 20 or 25% of 4 or 5 types of grains (wheat bran, wheat, barley and oat screenings and mixed milling dust) were compared to the control. All treatments led to similar fresh biomass productivity (≤10% variability). Diet with a higher carbohydrate (CHO) content did not affect larval CHO content ($P=0.4537$), but significantly increased lipid content (from 20 to 30%, $P\leq 0.0001$) and decreased protein content (from 60 to 50%, $P=0.0002$). Increasing dietary content of NDE, ADF and ADL increased protein content of larvae. This suggest that milling residue upcycling can both affect total biomass yield and nutritional value of mealworms, which is pertinent for the development of a circular economy model where mealworms would be incorporated in chicken feed.

Characterisation of penicillin bio-degradation in solid waste treatment by *Hermetia illucens* L. larvae

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As one of the most common antibiotics in the process of livestock and poultry breeding, penicillin G sodium (PEN) cannot be completely metabolised in animals and released into the environment as urine or faeces. The residual antibiotics will damage human health and destroy the ecological environment. Gradually, the insect technology -black soldier fly larvae (BSFL) has efficient degradation ability of antibiotics in livestock and poultry faeces. Here, we demonstrated that BSFL could degrade PEN with its intestinal microbes. After 12 days of treatment, the degradation rate of PEN mass was more than 90% by BSFL. Five pen-resistant strains were isolated from the larval intestinal tract, which were BSFLG-LAC1 (*Morganella morganii*), BSFLG-LAC2 (*Bacillus velezensis*), BSFLG-LAC3 (*Bacillus subterraneus*), BSFLG-LAC4 (*Trichophyton mentagrophytes*), and BSFLG-LAC5 (*Lichtheimia corymbifera*). The degradation efficiency of BSFLG-4 intestinal microorganisms was the highest, reaching 79.94%. This paper provides a new idea to eliminate PEN in livestock and poultry manure.

Limitations in the usage of organic waste in black soldier fly large-scale insect rearing systems and research relevance

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In a world with an ever-growing population, the generation of organic waste remains an important concern. Every year 1.3 billion tonnes of food – one-third of the worldwide farm-to-fork production – ends up as waste as per the traditional ‘take-make-waste’ linear economic model. The migration towards a circular economic model is required to close the gap between the ‘waste’ and the ‘take’, thus redirecting the waste back into the production cycle. The larvae of *Hermetia illucens*, also known as the black soldier fly, are currently produced in mass-rearing systems. Due to their capacity and flexibility to grow in a variety of substrates, black soldier fly larvae offer a sustainable solution to recycling organic waste and converting it into a high protein and fat ingredient for animal feed. However, using waste as feedstock for insect rearing can be limited by several factors. Firstly, the location of the rearing facility can determine the types, frequency, and availability of organic waste that is included in insect feeds. The lines between what is defined as waste and what is defined as animal feed vary spatiotemporally and therefore need to be evaluated at a local scale. Another limitation is the variability in the nutritional profile of the waste. Highly variable feedstocks lead to constraints in feed formulation, forcing producers to search for alternative ingredients and therefore increase production costs. Feedstock variability may also decrease biological stability throughout the rearing process leading to fluctuations in product quality. Lastly, the physical-chemical characteristics of the waste could also introduce limitations. Different substrates require added costs of processing and could generate constraints in downstream operational processes. The challenge for our industry is to discover ways to innovate the inclusion of diverse organic streams by providing a consistent and available feedstock supply that meets the nutritional needs of insects as well as the goals of insect-based product production.

Mechanisms of ciprofloxacin degradation by black soldier fly larvae with associated intestinal microorganisms

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Antibiotics are challenging to degrade and are excreted by livestock which results in environmental pollution. In this paper, we demonstrated that environmentally friendly manure bioremediation performed by black soldier fly larvae (BSFL) is a wise alternative, which could effectively degrade ciprofloxacin (CIP) by approached 85.48% in artificial diet and 84.22% in poultry manure within 12 days. They are up to 2.5-4.0 fold more than that achieved by natural fermentation. The five CIP-degrading strains were isolated from the larval gut, two of which, named by *Klebsiella pneumoniae* BSFLG-CIP1 and *Proteus mirabilis* BSFLG-CIP5, could degraded CIP by nearly 98.22 and 97.83% *in vitro*, respectively. When the intestinal isolates were re-inoculated to sterile BSFL system, the degradation level significantly increased up to 82.38%, comparing with the sterile BSFL system (21.76%). It is proved that the larvae intestinal microbiota might carry out this highly-efficient CIP-degradation. Furthermore, seven possible metabolites were identified for CIP-degradation *in vitro*, and they were referring three main potential degrading mechanisms of hydroxylise, piperazine ring substitute and cleavage, and quinoline ring cleavage. In conclusion, the present study may provide a strategy to reduce antibiotics pollution in animal waste through bioremediation with BSFL and adjusted intestinal microbes.

Gas emissions from food waste conversion process by using black soldier fly larvae (*Hermetia illucens*)

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Using black soldier fly to convert food waste to produce insect protein and oil has become a research hotspot. However, there are few studies on the characteristics of gas emission during the conversion of food waste by black soldier fly larvae. Besides, it was reported that there were huge differences in NH_3 emission when it was evaluated in Lab-scale and Industrial-scale. In this study, food waste was used as the main feed for black soldier fly larvae in production-scale, and the effects of four production conditions on NH_3 and greenhouse gas (CH_4 , N_2O and CO_2) emissions were investigated. The results showed that the production of NH_3 could be significantly inhibited by increasing water content, as well as the C/N of food waste and feed depth, and the total emission of NH_3 decreased by 53.77, 61.95 and 42.81%, respectively. The total emission of NH_3 increased by 54.33% with the increase of larvae density. The emission of NH_3 caused 1.87 to 12.11% of nitrogen loss in different conditions. The initial characteristics of food waste have negligible effects on the emission of CH_4 and N_2O . The daily emission of CO_2 is closely related to the growth of black soldier fly larvae. The lower C/N of food waste in the range of 10-30 could promote CO_2 emission during the transformation process. In this study, and the properties of the initial feed were reasonably controlled by adjusting the moisture content, C/N, larvae density and feed depth. The gas emission features by black soldier fly larvae during the conversion of food waste was revealed, which can provide theoretical support for gas pollution assessment in the breeding process of black soldier fly.

A novel BSF production technique for outdoor waste treatment

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This abstract provides a high level assessment on the feasibility of using an outdoor black soldier fly (BSF) waste treatment technique for the commercial production of larvae in tropical, subtropical and mild temperate climates. This work holds significant value due to its implications on the accessibility and affordability of scaled BSF production, particularly within developing economy and resource constrained contexts. The work was conducted in a commercial BSF production plant of Nambu Group (Pty) Ltd in South Africa. The trials were done between the months of June to November 2021 (mid winter to mid summer), where compost rows of varying sizes were established and monitored over the 6 month period. Data collection was focused on outdoor temperature range, intra-waste temperature range, feed moisture content, larval growth, final larval yield and feed composition. Production was recorded in a range of outdoor temperatures between 8 °C (± 46 °F) and 35 °C (± 95 °F) on waste treatment volumes ranging from 100-500 kg per trial. These figures were then compared to a control sample of indoor containerised production with the same feeding ratios, feed mixture, larval densities and feeding depth. Comparative performance was noted between the indoor and outdoor approaches with an average larval weight of 0.212 g produced outdoors and 0.239 g produced indoors. The outdoor technique resulted in slightly lower yield of larvae p/kg feed given (approx. 22.2% conversion), whereas the control resulted in an average conversion of approx. 23.7%. This difference was largely attributed to predation and crawl-off. Findings indicate that under suitable climatic conditions and with some basic infrastructure and equipment, it is feasible to produce BSF economically and at scale in an outdoor setting. This does require a set of parameters be met and suitable mitigation strategies provided against crawl off and predation. This approach reduces the need for often costly indoor production space and waste treatment containers, which are expensive to purchase and cumbersome to handle and maintain. The implications of the work done are particularly promising for emerging BSF producers from the African Continent, but are equally applicable to other developing regions.

The emission of greenhouse gases and ammonia during the processing of organic substrates by black soldier fly larvae

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Organic waste management methods are accompanied by the release of different amounts of various gases into the atmosphere. This study focuses on the processing of 2 types of organic substrates using *Hermetia illucens* larvae (BSFL) compared to traditional waste management methods such as landfill and composting. Mixture of food waste and food production sludge were chosen as the model substrates. Determination of carbon dioxide was carried out on a stationary installation of A.N. Severtsov Institute Ecology and Evolution (Moscow), which was connected to a portable field system EGM-5 (USA). To determine methane and measure nitrogen, gas phase samples were collected using a syringe. The concentration of gases was determined on a Kristall-2000 chromatograph. The content of the sum of soluble ammonia and ammonium ion was determined on a photocolormeter. The results of the experiments showed that the processing of organic substrates by BSFL is a promising waste treatment method that can significantly reduce greenhouse gas and ammonia emissions compared to traditional waste management methods. The period of processing by larvae is 8-10 days, and is much shorter than such methods as burial and composting. In addition, it is possible to obtain up to 25% of the mass of larvae from the initial mass of waste. The biomass of larvae after additional processing can be of value as a protein component for animal feed, and frass as an organic fertiliser. The main gas product when using BSFL technology is carbon dioxide, which is released during the respiration of larvae and microorganisms. Methane and nitrous oxide have been observed in minimal concentrations. The low layer of the substrate and the active movement of the larvae lead to a reduction in the anaerobic zones, which is the main reason for the insignificant emission of methane.

Multicriteria overlay to identify favourable regions for black soldier fly larvae bioconversion worldwide

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Globally, stakeholders are considering where to pursue black soldier fly larvae (BSFL) production for waste treatment and animal feed production. However, to date, little is known about the most favourable production locations. Based on available literature, correspondence with sector stakeholders (e.g. researchers, technology providers, insect producers), and the authors' knowledge, parameters identifying suitable locations include climate, availability of raw materials, infrastructure and utilities, labour costs, an enabling business environment as well as access to feed/fertiliser markets. Because priorities for these parameters vary with production scale, we identified regions most suitable for BSFL production for three different scenarios: smallholder production (household), medium-scale production (several tons feedstock per day) and large-scale production (>100 tons feedstock/day). Suitable regions were identified by overlaying geographical data in R/GIS with different criteria per scenario. Apart from labour cost and the ease of doing business index, only parameters which could be estimated on a subcountry level (e.g. 50×50 km) were used. These included temperature and humidity (CHELSA data) and the production of municipal solid waste (MSW, World Bank), animal manure (cow, pig and poultry, Gridded Livestock of the World) and agricultural by-products (104 crops of cereals, fruits, vegetables, oil crops, roots and tubers). Africa, Asia followed by South America have best environmental conditions (by area) for small-scale BSFL production. In contrast, climatized large-scale BSFL farms are more climate independent but rely on high-density feedstock production. Areas of high (>5,000 tons/km² × year) MSW, manure and agricultural by-product production were most abundant in India, Southeast Asia, Brazil, Argentina and Europe (by area). Tropic and subtropical regions are most suitable for medium-scale facilities due to a favourable climate and lower labour costs. Findings of this research support stakeholders in identifying favourable regions for in-depth analysis for BSFL production.

Intensified utilisation of BSF larvae for conversion of mycotoxin-contaminated maize into valuable protein sources

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Mycotoxin contamination of maize in Africa is a severe problem, exhibiting strong adverse health effects, especially regarding aflatoxins. Therefore, high percentages of the harvest cannot be regarded as safe. Contaminated maize kernels that are not suitable for animal or human consumption can be reintroduced into the feed and food chain with a higher value of protein. This can be achieved if the contaminated maize can be used to rear black soldier fly (BSF) larvae, without the contaminants being accumulated by the insects. This could improve the worldwide expensive animal protein and ecological challenges related to its production. However, whether this approach will optimise nutritional value, efficiency, and sustainability of food production and processing is still under investigation. In this project, young larvae (1 and 5 days old) were fed on naturally contaminated maize from Kenya. Clean maize and market waste were used as negative control while 25, 50, 75, and 100% contaminated maize were used as interventions. The suspected maize contained aflatoxins, fumonisins, deoxynivalenol, and zearalenone. Preliminary results indicated high survival of larvae with higher weight gain in mixed market waste than in the 100% clean maize group. Data collection and analysis are ongoing with great hope that this research will contribute to better quality animal feed and bridge the existing animal protein deficit.

Sustainability aspects of waste-based small-scale insect rearing

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Food security and nutrition are among the global goals of sustainable development (SDG 2). In view of the growing demand for food, the observed effects of climate change and declining natural resources, it is necessary to expand the range of food produced and reduce the amount of agricultural waste. One of the solutions in this regard seems to be new agricultural production systems focused on mass production of alternative organisms such as insects and alga, which have the potential to use nutrients from agricultural by-products and waste for their growth and development. They can convert different agricultural feedstock into protein and fat, including agriculture-based residuals that currently are discarded as waste. The paper discusses and evaluates the economic, environmental and social aspects of the small-scale insect rearing. The basis for the analysis was the assessment of a hypothetical production system in which agricultural residues are converted into dried protein product, using *Hermetia illucens* larvae (BSFL). The whole production process was modelled and simulated data were used for techno-economic analysis. Finally, the sustainability aspects of small-scale insect rearing were discussed. Mass flow analysis on a per-batch basis of 18 days' cycle showed different production effects depending on the substrate. The greatest yield of larvae was obtained on food waste, followed by potato peel and wheat straw. For the mentioned substrates, the final dry weight of the larvae amounted to 193.0, 169.5, 157.5 kg/batch and the protein content to 91.1, 81.5, 69.1 kg/batch, respectively. The simulated results of the economic analysis showed that the investment in BSFL production was profitable in conditions of feeding food waste or supplying a high value protein product.

Inclusion of 21 by-products for rearing *Hermetia illucens*: microbiological analysis

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The valorisation of by-products does not neglect food safety, but the implications have mainly been studied on single by-products. This study presents the bacterial profile of *Hermetia illucens* (HI) larvae and their rearing substrates resulting from the use of 21 industrial by-products stored as production waste by 12 firms. Six isoproteic, isolipid and isoenergetic diets and one control diet (GA–Gainesville) were used for a growth trial. The concentrations of staphylococci, enterobacteria, lactic acid bacteria (LAB), and aerobic and anaerobic sporigens were assessed in HI larvae (1.000 larvae/replica) and their rearing substrates (three replicates/diet) at two times (t_0 =6 life days, t_1 =13 life days). Analyses were conducted by plate counting and data were analysed by ANOVA and Tukey's post hoc test ($P<0.05$). At t_0 , both larvae and substrates showed initial bacterial contamination. The initial concentration of staphylococci (2.5–6.2 log cfu/g) increased in all substrates except Diet2, which had an acidic pH (3.3). The highest concentration of enterobacteria was detected in GA (4.17 log cfu/g) – due to their ability to colonise wheat bran (50% GA) – and increased in all diets as a consequence of larvae addition (3.8–8.8 log cfu/g). The initial concentration of LAB was high in substrates (4.3–6.1 log cfu/g) and larvae (5.6 log cfu/g) and tended to increase in both substrates (5.4–8.8 log cfu/g) and larvae (5.7–8 log cfu/g). Aerobic sporigens increased in both larvae and Diet1 and GA substrates due to the potential presence of cellulolytic bacteria associated with the degradation of draff (33% Diet1) and wheat bran (50% GA). Low concentrations of anaerobic sporigens were found in all substrates (1.7–2 log cfu/g), except Diet1 (3.2 log cfu/g). Their concentration also increased overtime after the addition of larvae which may be due to the increase in pH promoted by the larval metabolism. Differently, the initial high concentration in larvae (5.6 log cfu/g) decreased systematically overtime (2.7–5 log cfu/g). The use of industrial by-products has an impact on the presence and levels of contaminants and requires the adoption of new management, control, and stabilisation practices (e.g. UV light) for by-products, as well as ad-hoc regulations.

Cellulose decomposition from the livestock manure by *Hermetia illucens* L. (Diptera: Stratiomyidae)

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In a previous study, it was found that co-digestion of 40% dairy manure and 60% chicken manure with black soldier fly larvae treatment had a higher conversion efficiency, and better degradation effect on cellulose. Therefore, in this study, we compared cellulose hydrolysis, nutrient reduction, physico-chemical characteristics and conversion effects between the mixed manure (40% dairy manure and 60% chicken manure) and 100% dairy manure, studied the microbial communities in the larval intestine and manure by high-throughput sequencing, and revealed the differences of microbial communities related to cellulose degradation between the mixed manure and 100% dairy manure. This study solved preliminarily the problem that dairy manure is difficult to degrade due to its high cellulose content, and obtained high-quality insect feed and biological fertiliser, which laid a theoretical foundation for large-scale treatment of livestock manure.

Effect of six isoenergetic, isonitrogenous, and isolipidic by-product-based diets on larvae performance

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Agri-food by-products are generally poor in nutrients, but their combination can be optimised for black soldier fly (BSF) breeding through substrate formulation. The present study aims to evaluate the effects on BSF larvae growth of six isoenergetic, isonitrogenous, isolipidic (III) by-product-based diets (D1-D6) formulated using a combinations of 20 waste. The Gainesville diet (GA) was used as control. A total of 4,200 BSF larvae were distributed to 7 treatments (6 replicates/treatment). BSF larvae performance were determined by weighing 30 individuals/replicate every 4 days (T1: 10 day old; T2: 14 day old) until the box reached 40% of prepupae. At larval stage end, larva-prepupa time duration was calculated. Data were analysed by general linear mixed model. Considering the overall larvae growth, GA showed the best results followed by D1 and D5, while D2 performed worst ($P < 0.001$). No differences were observed between D4 and D6 ($P > 0.05$). Differently, D3 was statistically different from all groups ($P < 0.001$). At T1, GA and D2 maintained the best and the worst weight. Among the III diets, D1 was the greatest, and no differences were observed between groups D3-D5 ($P > 0.05$) and D4-D6 ($P > 0.05$). At T2, all treatments were different from each other, except for D1 and D3 ($P > 0.05$). The weight ranged from 0.096 to 0.228 g, and diets were listed from lowest to highest weight as follows: D2, D6, D4, D3 and D1, GA, D5. The longest development time was recorded for D2, while the shortest for GA ($P < 0.001$). The D3 reached faster the prepupa stage than D4 and D6 ($P < 0.001$). D1 and D5 showed an intermediate result between D3 and D6 ($P > 0.05$). In conclusion, although diets had a comparable nutritional composition, BSF larvae growth was influenced by other variables. Since by-products are extremely dissimilar from a physical point of view (particle size and water holding capacity), it is possible to hypothesise that the substrate texture affected performance and development duration. The study was founded by Fondazione Cariplo (CELLOW-FeeP).

POWERFOOD: enhancement of thermal energy from biogas for the integrated production of proteins

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Biogas and biomethane are pivotal points of the sustainable and circular economy development. Italy is a key player of the sector, with more than 1,600 production plants, of which 80% are in the agricultural sector, making Italy the second largest biogas producer in Europe. Biogas is produced *via* anaerobic digestion of organic materials such as manure, slurry, urban waste, agricultural biomass, agri-food waste and sewage sludge. Biogas is used in combined heat and power production, while the resulting material (digestate) shows interesting fertilising properties for agricultural applications. The process of biogas upgrading allows to obtain biomethane, a promising biofuel which can replace fossil methane. Biomethane will likely be used in the next years in the field of transportation, heating and cooling, as well as industrial uses. As a whole, biogas and biomethane allow not only the exploitation of renewable energy sources, with considerable savings of greenhouse gas emissions, but also the agricultural reuse of a by-product, with a reduction of mineral fertilisers. The POWERFOOD project, granted by the Regione Piemonte (PSR 2014/2020, Mis. 16.1.1) and implemented with the collaboration between two Universities and the Consorzio Monviso Agroenergia (gathering more than 150 biogas plants in agricultural context), aims to evaluate the feasibility of recovering part of the thermal energy generated by biogas plants to produce high-value protein materials. In particular, the goal of the project is the integration directly at farm level of the technologies for the production of insects' larvae (*Hermetia illucens*) and microalgae (*Arthrospira platensis*). Insects' larvae are gaining momentum as feed in the pet food sector, while microalgae such as spirulina already plays a key role in the field of food supplement. The activities of the project will range from the optimisation of the growing parameters in the two pilot plants, to the evaluation of the potential revenues coming from the production of these high added-value products.

Multifaceted approach to high fibre waste management using black soldier fly larvae

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With global food demands predicted to increase by 50% between 2020 and 2050, there will be a proportionate rise in the residual waste from food harvest which demands environmentally low impact strategies for management of such wastes. Black soldier fly (*Hermetia illucens* L.) larvae are being explored extensively as an economically viable solution for food waste management due to their ability to convert a wide range of low value substrates into high value animal proteins thereby simultaneously addressing the demand for animal feed. However, while several studies have assessed conventional waste substrates such as pre-consumer wastes and animal feeds, the residual waste from food harvest remains less explored. These wastes are high fibre substrates and have poor nitrogen to carbon ratio. Since black soldier fly larvae (BSFL) do not possess gut microbiota capable of breaking down lignocellulose effectively, the substrates need to be modified for consumption by BSFL. This study assessed the feasibility of different high fibre wastes, as feed, for BSFL. The impact of fermentation, inoculum and a modified feeding regime coupled with nutritional additives on palatability of the substrates is investigated. The fermentation or inoculum alone, as done in this study, is not sufficient to convert the substrates to a feasible feed. However, supplementing substrates with nutritive additives, at different time points during the rearing cycle, to assist larvae in digesting the main feed showed a 10-fold increase in bioconversion compared to control. The findings demonstrate that high fibre wastes are not indigestible to BSFL and require a multifaceted approach to enhance their nutrient availability to larvae thereby improving the rate of bioconversion.

Turning waste into protein-based materials by using black soldier fly larvae: the RICH project

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Waste valorisation plays a key role in supporting sustainable development and promotes the transition towards circular economy models. In this scenario, the use of insects as a primary agent for organic waste reduction and bioconversion into biomolecules could relieve problems related to its management and, at the same time, generate innovative biobased products, with environmental and economic benefits. The project RICH (Turning Rubbish Into biobased materials: a sustainable CHain for the full valorisation of organic waste) aims to develop an innovative and integrated circular economy chain, which, starting from the biotransformation of the organic fraction of municipal solid waste (OFMSW), allows the targeted production of biobased materials with high technological value and environmentally friendly, such as bioplastics and other advanced protein-based materials. The results obtained in the first part of the project demonstrate that BSF larvae were able to efficiently reduce OFMSW, as indicated by all the bioconversion indexes. Moreover, their protein and lipid content were 39 and 44% (DM), respectively. Following separation and purification, a similar crude lipid content was determined for larvae and pupae, i.e. $\approx 42\%$ (DM), while the relative protein yield by employing a pH-solubility method of extraction resulted in a significantly higher amount from pupae ($41.2 \pm 2.3\%$) than from larvae ($31.8 \pm 1.5\%$). Protein extracts obtained from both developmental stages were deeply characterised by means of proteomics, and physical and analytical techniques. SDS-PAGE revealed a wide distribution of polypeptides' molecular weight with values higher than 250 kDa. Both extracts, with the addition of plasticisers, were used to produce free-standing and flexible biofilms, thermally stable up to about 150 °C, with high quality, in particular from larval extracts. The study was funded by Fondazione Cariplo (2020-0900).

Heavy metals content in the *Hermetia illucens* larvae after various feed substrates processing

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The increased content of heavy metals in the *Hermetia illucens* larvae biomass can be a significant problem for using it as part of feed for animals farming. In this report, the heavy metals content in the larvae biomass was studied depending on different feed substrates compositions. The experiment took place at Entoprotech production plant in Penza region, RF. Wheat bran, food production sludge, vegetable mixture and mushroom waste were used as feed substrates. Feed substrates were inoculated with 5DO larvae with a density of 5 cm⁻². When processing was completed, the larvae were separated from the frass and dried using infrared complex. Part of the dried biomass was defatted using a screw oil press to produce meal samples, which were also selected for heavy metals studying. The prepared samples were tested for the content of nine toxic elements: Zn, Cd, Co, Mn, Cu, As, Ni, Pb, Hg, Cr. Statistical processing of the results of the study was carried out using Anova, paired comparisons were performed using the Tukey test. The results showed that the initial feed compositions significantly varied among themselves in the content of the main types of metals studied: Cd, Co, Mn, Cu, Ni, Zn. It was found out that the content of heavy metals varied significantly in meal samples produced from larvae using defatting process. Dry larvae grown on various feed substrates differed only in the content of Mn and Cu. The concentration of the remaining heavy metals in the studied samples was the same. Thus, the studied samples differed in the concentration of Cd, Mn, Cu, Zn, Cr and Pb, while the content of As, Hg, As and Ni remained the same. The accumulation of some researched elements by *Hermetia illucens* larvae after processing was observed. Thus, concentrations of Cd, Mn, Cu and Zn clearly accumulated from the feed substrate in meal samples. Finally, we checked using of sorbents (limestone and zeolite) to reduce the toxic elements content in the insect product. The obtained data showed the opposite result. The content of toxic substances growth up with increasing of concentration of the sorbent in the substrate.

Valorisation of waste by-products from olive pomace industry by using black soldier fly larvae as bioconverter

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The expected exponential growth of the population combined with economic development and rapid urbanisation will generate greater demand for food, with a consequent increase in the production of waste and by-products. Black soldier fly larvae (BSFL) can grow on a wide range of wastes and have a high nutritional value, making them a valuable source of protein for the feed industry as possible replacements for soybean meal and fishmeal. In the agri-food sector, the olive oil industry is penalised by waste disposal processes. The management of olive pomace (OP), a by-product of this industry, is one of the major environmental problems due to its highly polluting nature and its high disposal costs. The aim of this research was to evaluate the effects of OP as a component of BSFLs rearing substrates on their development and waste reduction efficiency (WRE). To evaluate the effects of OP on BSFLs, three different inclusions of OP in a commercial diet for BSFLs were compared by modulating the amount of OP in it (control: 0%, S1: 10%, S2: 20% of total). 2,000 larvae per replica were used to evaluate it and four replicates per treatment. Increasing the OP concentration in the rearing substrate had no significant effect on the growth performance of the BSFLs, despite the higher content of structural carbohydrates (e.g. lignin). Instead, the high amount of lignin in the OP influenced the WRE of the BSFLs: their capacity decreased as the concentration of OP in the substrate increased. An abnormal BSFLs mortality was found in a sample reared on a substrate with 20% OP. The cause of this unexpected mortality could be due to the persistence of a broad-spectrum pesticide in the olive grove pest management. OP can be used as a component of rearing substrates to breed BSFLs. The use of different percentages (up to 20%) of OP in the BSFLs rearing substrate can be considered as a valid solution for its utilisation and recycling, hence OP could be considered as a potential resource for the insect sector and not only as a waste product. Future research will be of interest to gain a fuller understanding of how pesticide residues in OP affect BSFLs.

Growth of black soldier fly larvae (*Hermetia illucens*) on heat treated supermarket waste

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Nowadays, about one third or around 900 million tonnes of the globally produced food is lost or wasted in the food supply chain, and about 13% of the food waste is originating from supermarkets. This supermarket waste can be upcycled by as substrate for rearing black soldier fly larvae (BSFL). BSFL can grow on a wide variety of organic materials and are able to convert low value substrates into high value biomass. Because of the possible presence of food pathogens in supermarket waste, a heat treatment is required to reduce harmful organisms and create a microbiologically safe substrate. Since this heat treatment can also have an detrimental influence on the nutritional quality of the substrate and potentially kill beneficial micro-organisms that promote the growth of BSFL, it should be evaluated whether the larvae can still thrive well on heat treated supermarket waste. As part of the FACCE SURPLUS UpWaste project, BSFL were reared on supermarket waste. This supermarket waste was created by standardised mixing of foods (without meat or fish) from a supermarket. A heat treatment of 60 °C for 10 minutes was applied in order to result in a microbiological safe substrate (as shown in previous research). BSFL were reared either on freshly prepared, untreated supermarket waste and on heat treated supermarket waste. Larval performance and bioconversion were measured during the experiment. These experiments (which are currently being performed) will reveal whether BSFL can grow well on heat treated supermarket waste and whether differences in growth performance occur between the untreated and heat treated supermarket waste.

Processing side streams from the agri-food chain with black soldier fly (*Hermetia illucens*) larvae

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At every link of the agri-food supply chain organic side streams can arise. Some plant parts never leave the field (e.g. brussels sprout stalks) as there is no current market for those products and others are unsuitable to be marketed (e.g. tomatoes with blossom-end rot). Further down the chain, some side streams occur during processing (e.g. cheese whey, potato peels) and some arise due to a limited shelf life in retail. Larvae of the black soldier fly are often proposed as efficient converters of low grade biomass into high grade biomass (proteins and fats). Previous research gave an indication of the required structure and water retention of feeding substrates suitable for rearing black soldier fly larvae as well as their nutritional requirements (25-35% dry matter (DM) and 10-20% protein on a DM basis). However, not all side streams are equally nutritious and these need to be properly mixed, preferably with other side streams, to get a suitable rearing substrate for the larvae. Within the European H2020 project 'Susinchain', a standardised protocol for feed trials was developed and validated. This protocol was used to validate different side streams, which included leek foliage, Brussels sprout stalks, cheese whey and unsold leftovers from a local retailer (a mixture of fruit and vegetables, bread and dairy products). Experimental conditions were the following: ambient temperature was 27 °C, 60% relative humidity and 15,000 larvae per crate of 60×40 cm. Gainesville housefly diet was used as a control feed. The initial results indicate that BSF larvae can survive on most substrates albeit with variable success. In the next step an economical assessment has to be made to pinpoint the most cost effective feed.

Characterisation of hatchery residues to implement pre-treatment and black soldier fly larvae bioconversion

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Black soldier fly (BSF) larvae have the potential to efficiently bioconvert hatchery residues while generating sustainable alternative feeds. However, the management of hatchery residues bears feed safety and animal health issues. The application of a pre-treatment will be necessary to stabilise these residues and reduce the risks associated with their use. However, variations in the composition and storage conditions of hatchery residues will influence their nutritional value and microbiological loads and thus, the pre-treatment process. The present study aims to characterise the nutritional and microbiological quality of hatchery residues and to identify the critical values over a one-year period. To achieve this, samples of hatchery residues (125 g), stored in a silo for a maximum of 24 hours, were taken every two weeks from May 2021 to April 2022. For each sampling event, triplicate samples were analysed for proximate composition, pH, colour (L*a*b*, Chroma) and microbiological loads (total mesophilic aerobes (TAM), enterobacteria (ENT), lactic acid bacteria (LAB)). Principal component analyses were applied to visualise the interactions between these parameters. From May to December, despite significant variations with time, all residues were found to be rich in energy (2,498.0 to 5,799.87 Cal/g, DM), protein (21.28 to 43.65%, DM) and fat (14.55 to 28.61%, DM), but low in carbohydrate (0 to 15.25%, DM). The ash content is highly variable (12.32 to 49.14%, DM) and depends on the eggshell content. Microbiological loads are high for TAM (7.06 to 8.79 log cfu/g), ENT (6.30 to 8.55 log cfu/g) and LAB (6.65 to 8.93 log cfu/g) based on Quebec provincial guidelines for raw poultry and egg products. The determination of critical thresholds for residue quality will allow us to identify suitable pre-treatment processes aiming to store and improve hatchery residues and their bioconversion by BSF larvae. This project will provide data for the accreditation of insect products fed with animal co-products in Canada.

Valorisation of pretreated biogas digestate with black soldier fly (*Hermetia illucens*) larvae

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Increasing concerns related to the negative environmental impacts of food waste has motivated the development of new solutions to complete the organic waste cycle. One particular waste product, the solid digestate from anaerobic digestion, has been identified for further bioprocessing. Black soldier fly (BSF, *Hermetia illucens*) larvae are known for their great potential in the processing of organic waste. In this study, this potential was investigated to further process digestate waste stream. Digestate is seen as a relatively low nutrient source for BSF. However, the lignocellulosic matter in this residue could be enzymatically hydrolysed to release residual carbohydrates. For this study, digestate from a full-scale anaerobic digestion plant in Quebec (Canada) which processes a range of feedstocks: fruits, vegetables, garden wastes, sludge derived from dairy processing and wastewater was used. Digestate was treated with Accelerase® DUET enzyme complex to hydrolyse lignocellulosic matter and compared to a standard diet. 600 four-day old larvae were fed daily with 160 g (70% relative humidity) of diets for 6 days and harvested 3 days later. Although their growth and total biomass were significantly lower than the standard diet, larvae fed on hydrolysed digestate were almost two times larger than the larvae fed on crude digestate. Furthermore, the content of organic matter, lipids and minerals in the diets and frass were analysed. According to this study, enzyme-treated digestate does not allow efficient larval growth compared to the standard diet. The development of a more effective method of pretreatment is required for BSF larvae to become an eco-friendly solution for digestate valorisation.

Effects of different larval densities of the black soldier fly in two types of livestock manures

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The use of black soldier fly (BSF) for biowaste processing is increasing in Brazil. However, the importance of some rearing factors, such as larval density, have been overlooked. Most studies with different organic wastes are performed only with a single predetermined density, but the quality of insects produced and the final amount of frass and organic matter processed can be strongly influenced by the number of larvae inoculated. Therefore, this study aimed to determine the effects of larval density on life-history traits of the BSF in bovine and ovine manures. The BSF eggs were obtained from the company Cyns® and seeded in a start diet made of chicken feed and coconut fibre, and, when the larvae were 5 day-old, they were inoculated in plastic trays containing 7 kg of each manure in a room at 27-32 °C. Five different seeding densities were tested in bovine manure (0.01; 0.025; 0.5; 0.1 and 0.2 g of eggs/kg of manure), while six densities were tested with ovine manure (0.01; 0.017; 0.025; 0.5; 0.1 and 0.2 g of eggs/kg of manure). The mean larval periods (until appearance of the 1st prepupa) were 8 and 7 days in the bovine and ovine manures, respectively. In overall, our results showed an increase in larval mass at low densities and higher reductions of the manure mass at high densities in both substrates. In the bovine manure, the mean individual prepupal weight reached 254±82 mg with a 48% reduction of manure weight at the 0.01 density, while the 0.2 density allowed a 64% reduction in manure weight but larvae weighting up to 117±27 mg/larva. In the ovine manure, at the 0.01 density the mean final larval weight was 163±25 mg/larva with a 39% reduction of manure weight, whereas the 0.2 density allowed a 49% reduction in manure weight but larvae weighting 79±19 mg/larva at the harvest moment. In conclusion, if the consumer is interested in obtaining good quality larvae for mass rearing purposes, low seeding densities of BSF eggs can be applied in both manures, but if the target is only to reduce the organic waste as much as possible, high seeding densities should be used.

The window of accessibility, a framework for bioconversion with *Hermetia illucens*

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Hermetia illucens larvae have been shown to have an incredible capacity to convert food waste into proteins and fertiliser. This process is governed by many biotic and abiotic factors. Here we show the impact of feed moisture content, which makes food available to larvae, larvae size, which plays on their ingestion capacity, and population density on their bioconversion capacity. The interaction of feed availability and the total feed ingestion capacity (ingestion capacity and population density) is shown to be the determining factors regulating bioconversion. We propose a general framework based on these factors to better elucidate the synergy and complexity of rearing larvae in their substrate. This framework enables us to maximise the bioconversion capacities of larvae when the food moisture content is sufficient, and to explore ways of keeping moisture content at sufficient levels. The mechanisms suggested by this framework are directly applicable to the good functioning of industrial insect rearing.

Improving *Tenebrio molitor* rearing performance and frass composition using carrot or mill residues:hemp meal diet

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Mealworms are a new nutrient source for humans and are also considered for upcycling food waste (FW) thus contributing to decrease global use of agricultural feed. This study aimed to measure the impact of 5 FW on keys performance indicators: biomass, larvae and frass composition, feed reduction (R), efficiency of conversion of ingested food (ECI). To do so, 4 wk-old larvae (2 larvae/cm²) were fed in two separate experiments (4 replicates each) with 0.8 g of diet on dry basis with a water source uniformly dispersed (0.04 ml/larva) twice a week for 10 weeks. The first experiment compared three FW provided as water source: carrot (C), potato (P), or apple pomace (A). Diet including C produced a greater biomass followed by P and A (16.7±1.7, 14.5±1.6 and 10.9±1.6 g, respectively). Watering with C and P resulted in a greater feed reduction of 21.3±4.9 and 22.2±3.1% compared to A (11.6±3.8%) stressing the importance of nature of the water source on efficiency. Since the FW water sources were organic matter, it impacted the frass composition. Indeed, C and P resulted in a lower carbon to nitrogen factor (C/N) compared to A (22.5±0.5, 22.9±0.4 and 25.2±1.4 respectively). The second experiment used C as water source but compared different ratios of mill residues:hemp meal (75:25, 50:50 or 25:75) to a reference feed (RF; bran:corn; 1:1). Interestingly, the protein content of larvae fed with mill residues: hemp meal treatments produced larvae with higher protein content (37.3±2.2%) and lower lipids extract (34.4±2.5%) compared to the RF (34.1±2.6%, 41.5±1.6% respectively). The 75:25 diet showed larvae weight and ECI, similar to RF showing that FW can replace agricultural feed without affecting rearing performance. Moreover, the mill residues: hemp meal treatments generated frass with higher mineral content compared to RF which increased by two-fold their value as fertiliser. This work provides key findings supporting that high quality larvae and frass can be produced using food waste as water source and/or diets for *Tenebrio molitor*.

Modelling utilisation of typical past due protein-rich waste streams by black soldier fly at a semi-industrial scale

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Protein-rich waste generated by food industry may be valuable feedstock for black soldier fly (BSF) larvae. To explore the feasibility of processed meat and dairy past-dues for potential enterprise, we analysed waste stream daily output in a typical logistic facility and designed several potential diets with waste content of 33 or 40% by wet mass complemented with bran and potato as structuring agents. The diets were evaluated at an R&D scale in a climate-controlled facility with daily assessments of larvae growth dynamics, substrate temperature and humidity and detailed nutritional and physico-chemical analysis of the substrate and end products. The bioconversion yield on all diets tested was above 22% with some compositions demonstrating average efficiency above 25%. One of the compositions was tested at a semi-industrial scale (1 ton) with bioconversion efficiency identical to the R&D processes. To conclude, protein-rich wastes are highly suitable for the nutrition of the BSF larvae at the industrial setting with good humidity and temperature range, excellent bioconversion and harvesting characteristics. Moreover, due to high bioconversion rate, recycling by BSF may unlock a highly efficient environment-friendly means of utilising meat waste for non-productive applications.

Influence of different suboptimal feeding regimes on the performance of black soldier fly (*Hermetia illucens*)

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Among insect species reared as alternative protein sources, *Hermetia illucens* (black soldier fly, BSF) has shown a huge potential mostly due to its high protein content, its bioconversion rates and versatility in using different feeding substrates. As with traditional livestock production, insect rearing uses different feeding input regimes, mainly continuous or batch feeding. Among the used substrates supermarket feedstock waste gained recent interest as a form to recover lost nutrients, under a circular economy framework. However, several uncertainties remain regarding the heterogenous nature of these substrates. This experimental work focuses on continuous feeding system and the potential effects of suboptimal feeding under different conditions of temperature and substrate moisture that may occur between food supply events. To assess this, we used seven different isolated vegetables as feeding substrates (wheat, pumpkin, apple, pomace, red onion, red cabbage, spinach), and evaluated BSF performance and bioconversion parameters (biomass weight, survival rate, waste reduction index, bioconversion rate, feed conversion rate and efficiency of conversion of digested food). Our results showed that some substrates should be avoided when rearing *H. illucens* on feedstocks. Among these, apple led to poorer and slower development performances, while pomace and spinach showed high mortality rates indicating that there probably some anti-nutritional compounds. The substrates in which BSF presented good performances were, pumpkin, red cabbage and red onion. The knowledge regarding the use of feedstock to feed insects and recover important nutrients is paramount to achieve a true circular economy framework, in line with United Nations Sustainable Development Goals. This work delivers relevant results for black soldier fly reared on continuous feeding system using vegetable feedstock substrates and their potential trade-offs.

Safety and bioconversion efficiency of aflatoxin contaminated maize by black soldier fly larvae

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Globally, 25% or more of food crops, and in Rwanda, 65% of maize are lost due to contamination with mycotoxins. Previous lab/bench-scale research with spiked feedstocks suggests that black soldier fly larvae (BSFL) convert aflatoxin contaminated agri-food side streams into safe nutrient-rich animal feed. In this research conducted in Kigali, Rwanda (completed by May 2022), we study feed safety and BSFL bioconversion efficiency of naturally and highly aflatoxin-contaminated (~400 mg/kg) maize at a pilot-scale bioconversion facility with the aim to establishing a commercially viable and safe reuse option for mycotoxin contaminated agri-food side streams. Aflatoxin contaminated maize will be separated from clean maize with an automatic sorting machine. Following, three different aflatoxin contamination levels (25, 50, 100% of contaminated maize) will be produced by mixing contaminated with clean maize. The maize will be then milled and mixed with organic waste at three different levels (40, 60, 80% maize of total BSFL diet based on dry mass) to identify a commercial BSFL diet. Using typical commercial procedures (e.g. bin size 60×40 cm, density 4 larvae/cm²), all diets will be fed to BSFL in controlled feeding experiments. Larval weight, bioconversion rate, feedstock reduction as well as environmental parameters will be recorded, and larvae and frass analysed for nutrient composition. The safety of the larvae will be assessed by comparing analyses results of mycotoxins and degradation products (AFB1/2, AFG1/2, AFP1, AFQ1, AFM1, Aflatoxicol) to animal feed standards. Findings from this study will be shared during the conference.

Insect production systems/optimisation, engineering, automation & security

Influence of substrate protein content on *T. molitor* larvae composition and determination by near infrared spectroscopy

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Near infrared spectroscopy appeared as a rapid technique for simultaneous quantitative determination of numerous nutrient parameters and is used for process and quality control of feed and food. The yellow mealworm (*Tenebrio molitor*) is an edible insect and an auspicious candidate for breeding and production on industrial scale. In order to provide a balanced feeding or to achieve a favoured larval composition, it is necessary to monitor and regulate insect feeding. In our precedent studies, we could ascertain that nutrient composition of the feeding substrate has an effect on larval composition, especially on protein and amino acid content. The current experiments represent a new advance to consider changes in the protein content of *T. molitor* larvae using NIRS combined with multivariate analysis. Living larvae were scanned with a near infrared spectrometer using wave lengths from 1,100 to 2,100 nm. Various feeding substrates with different protein and amino acid content were tested and the impact on larval composition was measured. Spectral curves were correlated with larval protein content analysed by analytical standard methods. A calibration was evolved, using MATLAB and PLS toolbox, with modified partial least squares as regression method. It could be shown that NIR spectra were influenced by composition of feeding substrate and larvae, since the absorbance of the larval groups differed greatly. Protein and amino acid content of the feeding substrate had a significant influence on the weight gain and development of larvae. As a significant result, differences in larval composition could be determined, depending on feeding. With respect to wet weight of the larvae, protein content varied from 10.5 to 22.6%. These results show, that the composition of insects can be continuously recorded and calculated with NIR, so that specific feeding can be implemented as part of larval development. The next step is to build a robust calibration for industrial scale rearing of *T. molitor* larvae, in order to be able to develop process-optimised strategies for the best possible cultivation and processing of mealworm larvae.

Practical guide to commercial BSF farming

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Every day new entrepreneurs stand up wanting to start in the insect industry. They come with great enthusiasm looking how they can start this new business. Assuming they can obtain the right knowledge they also get a reality check. Because it's not a business for a quick win. Like every other business you first need to realise who is your customer? Which sounds logic but I see on a weekly basis that it's not common. Is your focus on food, feed or waste? Do you want to produce as many insect protein possible? Or is your focus to process the largest pile of organic waste? Considerations where every new entrepreneur should be pointed out to. In my abstract the focus is on BSF farming with no focus on food, focus is on feed and waste processing. The feed industry is a cost price driven market with demand of high volumes. Challenge for the BSF farmer is that you can't gradually grow your business. It starts with a pilot facility (f.e. 2 tons of wet waste per day) and there you make the step to f.e. 50 tons of waste per day. So from the moment you start, also with a pilot, no matter if you use trays or ZOEM racks. Figure out in advance your next steps. This again sounds logic. But when I started I've seen many examples of farmers who started with trays but after 2-3 years stopped because the plan on how it would look commercially was not taken care of and/or was not viable. That should be lessons learned. The commercial attractiveness of your BSF farm has of course many factors. Location, energy cost, available waste, cost price waste, sales market, etc. Some lessons learned I can share from Sanergy in Kenya. Who in practice today processes 160 tons of organic waste per day. Their focus is processing as much as possible organic waste each day. For the EU grower that focus is less attractive because the choice of which waste streams you're allowed to use is limited. At same time let that be a lesson for the decision makers in EU. A quick focus is putting as much as possible substrate per m² floor surface. Which in itself is not bad but everything has a tipping point. That is often a blind spot for the new to become farmer. Air circulation is very important in BSF farming and it also has that tipping point. Practical examples can be shared in Quebec.

Multi-objective integrated decision support tool for SUsustainable INsect CHAINS production

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Determination of insect production sustainability is a complex and challenging task. Life cycle sustainability assessment (LCSA) of insect production can estimate social, economic, and environmental impacts. When insects are used as feed and food, several aspects of sustainability must be additionally considered including food and feed production regulations, factors that affect nutrition, social acceptance, and regulation of greenhouse gases. The multi-objective integrated decision support system (DSS) based on: (1) modular LCA and scenario-based life cycle inventory superstructure databases that analyse the environmental, social, and economic performance of multiple insect species (*Acheta domesticus*, *Musca domestica*, *Hermetia illucens*, *Tenebrio molitor*); (2) analytic hierarchy process to evaluate and rank different insect production scenarios based on decision-makers; and (3) non-dominated sorting genetic algorithm to obtain pareto-optimal solutions covering all sustainability indicators to compare insect species production scenarios and to test the sensitivity of results with different feeds, processing and utilities, type of end product, packaging, and scaling options. The beta version of the DSS allowed to define that the most eco-efficient scenario in Europe was for house cricket (*A. domesticus*) reared on plant residues subject to blanching and microwave drying and packaged in polyethylene foil. *T. molitor* had the lowest carbon emissions despite the high non-renewable energy use compared to *H. illucens*. An online web-based multi-objective integrated decision tool can demonstrate its effectiveness in identifying hotspots in the insect production system. However, more data is needed. For the concept of insect production sustainability to be implemented on a large scale, environmental accounting methods that include holistic environmental, economic, social, and technical information are required.

Enhancing control and hygiene with a dark cage/love cage-system for a black soldier fly nursery

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The production of hatchlings in a nursery for black soldier fly waste bioconversion involves all life stages of the fly. Knowledge on preferences and needs of adult flies and how to integrate this into operations is key towards fostering successful mating with reliable oviposition and prosperous hatching. As adult flies are relatively fragile, staff handling with direct contact to flies bears the risk of flies escaping from the cages when opened by staff, or death when staff enter cages and step on resting flies. Another example is the increased risk of staff introducing contamination from outside such as diseases, parasites or parasitoids. To minimise these risks we suggest more non-invasive techniques operations. Another aspect is to align the life phases of the flies with the nursery tasks and to enable monitoring of key performance indicators (KPI; e.g. fly density, sex ratio, oviposition, hatching). Monitoring of KPI helps determining the source of defect in case of manufacturing errors. We present the rationale and experience when working with a dark cage/love cage-system in the nursery. It consists of a series of dark cages, containing pupae of the same age. The cages form a repository when adults eclose. Due to the lack of light in the dark cages, the flies remain inactive until they are attracted by a light source to fly through a tunnel into the love cage. Love cages are mobile and sequentially connected to the array of dark cages. This sequential operation ensures that: (1) the sex ratio in the love cage is well balanced; and (2) there are well defined and sufficient number of adults of very similar age in the love cage. This fosters synchronisation of mating and egg laying. The egg-laying peak can thus be clearly determined and all the eggs be harvested after a short time (4 days). After egg harvesting the love cage is dismantled and washed. By separating the individual steps, monitoring of KPI such as eclosion rate, mating rate or eggs/female is possible. In addition, there is no direct contact between staff and flies and the cages can be washed at short intervals, which prevents the spread of diseases.

Ready-to-use packages of suspended BSF neonates with extended shelf-life for decentralised and scalable insect farming

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Insects, and more specifically black soldier flies (BSF, *Hermetia illucens*), are an excellent alternative feeding source for farm animals as they have high nutritional value and benefits for the environment: they are efficiently grown in large masses, contain high nutrient values, grown on organic waste and providing a green food resource to support population growth. In order to make a true impact and replace existing components in animal feed, insect protein has to be produced at an industrial scale, all year long and in a stable manner. With current technologies, this is not achievable yet, as the life-cycle of insects is too short for them to travel great distances alive while preserving their development stage or integrity. Therefore, BSF protein factories today must maintain their own breeding colonies on site. This process is highly expensive, requires specialised knowledge, and is unreliable in terms of daily BSF egg output and defence faced with disease and colony collapse. We developed a system for reducing the neonate metabolism and maintaining their size and viability for two weeks. This suspended animation state is reversible as soon as the neonates are moved into regular feed. The larva growth analysis shows a high survival rate in good fitness compared to normally grown larvae. The technology utilisation is simple, works at ambient temperature, and is fit for industrial shipment of ready-to-use neonate packages directly to the rearing sites. This enables for the first time the decoupling of the breeding (reproduction) processes from the rearing (larvae growing) and processing steps in the insect farming supply chain and therefore creates a brand-new product in the market: ready-to-use insect eggs and neonates with extended shelf-life. Using the suspended animation technology, we can deliver pre-counted, ready-to-use packages of suspended BSF neonates with extended shelf-life. These packages can be shipped globally from one breeding hub to rearing and processing facilities located near organic waste concentrations used as larvae feed. These packages can be easily activated by deploying their content on organic waste feed, making the rearing process a simple and steady practice.

Evaluation of various feed additives and wet feeds for the development of *Alphitobius diaperinus* larvae

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The lesser mealworm, *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is among the insect species that are registered in EU as alternative protein feed sources [Commission Regulations (EU) 2017/893 and 2021/1372]. However, the success of its mass-rearing depends greatly on the optimisation of the dry and wet feed provided as growth substrate. Little is known though about the effect of protein sources as feed additives on the larval development of *A. diaperinus*. Similarly, limited information is available on the effect of different wet feeds provided as a moisture source on larval growth. Thus, the objective of the present study was to evaluate, in laboratory trials, the effect of various protein sources as feed additives, as well as different wet feeds as a water source on the growth and development of *A. diaperinus* larvae. In a first series of bioassays, wheat bran was supplemented with protein concentrates of pea, soy and brown rice, as well as linseed flour, brewer's yeast and baker's yeast as a protein source to form isoproteinic diets with 27.5% protein. Agar slices were provided as a moisture source. In another set of bioassays, larvae were grown on wheat bran and different wet feeds as a moisture source, i.e. carrot, potato, apple, agar and gelatine slices, as well as water crystals. Based on the results, the faster larval development (time to pupation) and highest survival rate were recorded when wheat bran was supplemented with brewer's yeast. However, larvae fed on bran with baker's yeast had the highest individual larval weight at the end of the bioassay. All wet feeds tested supported larval growth, with the faster larval development and survival rates being noted for carrot, potato and agar slices. These data aim to contribute to the optimisation of the mass-rearing of *A. diaperinus* to meet the industrial demands. This study is part of the project 'EntoFeed' (MIS 513644) that is co-funded by Greece and European Union under the 'RIS Innovation Call 2020'.

Optimisation of energy efficiency of industrial insect production with emphasis on climate control system (EntoPower)

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The production setups of BSF are all based on a similar design – semi-automated warehouses with stacked production trays. Controlling temperature and humidity during the production is of the outmost importance for a successful industrialisation. The mass rearing of BSF results in very high heat production and gas emissions. This puts immense requirements on ventilation and climate control which presently, to some extent, has been overlooked. In the EntoPower project, an innovative ventilation concept for BSF production, based on in-depth experience from other intensive animal farming, has been developed. The new heating and ventilation system aims to reduce the total energy consumption and increase uniformity in larval development. Main results of the project will be presented, including: (1) Piloting the ventilation concept: over a period of more than a yr. the ventilation concept has been developed and tested at a BSF-production facility. Continuous improvements have been incorporated into the concept, including a combined scrubber and heat recovery system. Moreover, during an extensive sensor-test, climate (temp., RH% and CO₂) and tray temperature in multiple positions was studied simultaneously providing greater resolution on climate uniformity. (2) 3D tray temperature profile: A point measurement of temperature in the substrate/tray gives little information on the development because the larval density may be unevenly distributed. As consequence, a 3D temperature sensor has been developed and patented. The sensor is continuously mapping the temperature profile of the tray in all three dimensions. (3) Substrate impact on heat and emission: Greenhouse gas emissions and heat development has been quantified using four different broadly applied feeding substrates. The heat development showed a similar pattern for all the tested substrates whereas large differences were observed in the gas emissions.

Effect of Bokashi fermentation on *Hermetia illucens* (Diptera: Stratiomidae) development and microbiome

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Fermentation technology can enhance the digestibility of the substrate for insects used to digest biowaste such as the black soldier fly (BSF) *Hermetia illucens* (Diptera: Stratiomidae) and lead to improved performance and higher protein yields, as shown recently for some bacteria evaluated singly, with other bacteria instead having a mostly detrimental effect. The Bokashi technology is a method for treating biowaste using controlled lactic acid fermentation under anaerobic conditions. So called 'effective microorganisms' (EM) consist of a mixed culture of microorganisms such as lactobacilli, yeasts and actinomycetes. When substrates are inoculated with EM the microbial composition shifts, and secondary metabolites that can perform beneficial functions are produced by the modified microbiome. Using a commercially available Bokashi starter, we anaerobically digested two organic substrates, fresh oranges and brewery's spent grains, with the Gainesville diet used a control. We aimed at assessing the impact of the fermented solid fraction on BSF development (weight, growth rate, developmental time from larvae to prepupae), on BSF protein content, on substrate bioconversion efficiency and on microbiome composition of both BSF larvae and substrates (before and after BSF digestion) at the industrial scale. Bokashi fermentation resulted in higher larval weight and reduced developmental time for BSF larvae that fed on Bokashi-fermented substrates. The effect was particularly remarkable on fresh oranges, showing the capability of Bokashi fermentation to increase the digestibility of an otherwise poor substrate. The effects of Bokashi fermentation on the microbiome composition of both BSF larvae and frass will also be presented.

Influence of temperature on some selected life history parameters of the African Palm Weevil larvae *Rhynchophorus phoen*

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The African palm larvae *Rhynchophorus phoenicis* (F.) (Coleoptera: Curculionidae) have been identified as a potential alternative to conventional protein sources from livestock and birds due to their rich nutritional content and the fact that they can be reared on agricultural by-products. In establishing a protocol for mass production of *R. phoenicis* larvae, the most crucial step is by understanding how temperature affects the egg, larvae, pupa, and the performance of adult during oviposition. To identify a suitable temperature condition for continuous production of the larvae, four temperatures (20, 25, 30 and 35 °C) were evaluated in an incubator at a constant relative humidity of 70% with a photoperiod of 12:12 (L:D) h on different substrates. The results revealed that the developmental duration of *R. phoenicis* larvae decreased with an increasing temperature above 30 °C whilst larval survival rate increased between 20 and 25 °C. The optimal temperature range for egg, larval and egg-to-adult development was observed at an average temperature of 25 °C. The extreme temperature with the fastest larval development rate and highest mortality was recorded at 35 °C. This means that facilities and containers used to produce *R. phoenicis* larvae should be designed to withstand a temperature of 25 °C.

The effect of variability on productivity

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The issue dealt with here is the rearing of an organism from egg to larva to pupa as is commonly done with various mealworms and black soldier flies, while organism development is the principal subject of interest. In order to obtain a uniform product at harvest time, the population should be synchronised as much as possible and this means that variability in development in the population must be reduced to the greatest possible extent. Four main sources of variability in development were identified. (Organism composition is not dealt with here.) Two of these are operational, i.e. due to the process. These are: (1) distribution of the initial ages of the eggs; and (2) distribution of incubation temperatures of the organisms. The two other sources are biological in nature; (3) inherent variability in the development rate of individual organisms; and (4) distribution in temperature sensitivity of the organisms (Q10 factor and inactivation rate). When combined, the impacts of some of these factors are convoluted. The effects of the various sources of variability on production and productivity were examined by means of simulation. In the paper it is illustrated how under a set of realistic reference conditions at harvest time the mix of larvae/pupae is 86/14% which can then be reduced to 95/5% by better regulating the temperature. This ratio can then be further reduced to 99/1% by narrowing the age distribution of the eggs. After that further improvements can be achieved by breeding the organism so as to synchronise their development. This kind of exercise is useful in a number of contexts: (1) during the very early stage of project planning, as part of preliminary project design, before any formal design work is started and well before any major financial commitments are made; (2) during trouble-shooting of a low-performing process in order to upgrade its product quality; (3) to develop specifications for egg age distribution, design specifications for incubation equipment, etc.; (4) to better understand what data are required about the organism of interest so as to facilitate process design; and (5) to set objectives for organism breeding efforts.

Impact of storage method on survival of early instar BSF larvae

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Black soldier fly (BSF) farming has gained attention in past years and optimising the production of small-sized larvae has, of late, been of interest. Previous studies have largely focused on optimising hatching substrates, temperature and relative humidity ranges in the nursery, egg traps, or fly density and lighting. Less focus has yet been directed to storing different life stages (larvae, pre-pupae or pupae) at temperatures below 16 °C for later use. Only a few studies have investigated the lower temperature threshold or the development of early instar BSF larvae at temperatures around 10–16 °C, with the purpose of discerning BSF larvae development or survival threshold but not cold storage at lower temperatures for longer periods of time has yet been studied. Preliminary results have shown that desiccation or chill damage all lead to losses in stored early instar larvae. Hence, the experimental setup was designed to slow larval growth by lowering the temperature while simultaneously providing moisture throughout the storage period with different means. The temperature in this study was chosen based on preliminary trials and on previously established lower-temperature thresholds from literature. We investigated the survival rate of two sizes of larvae (<1 mg and >1 mg) for 2 durations (7 and 14 days) at a constant storage temperature of 12.5 °C with different inclusion rates (33%, 50%, 66%) of moistened wheat bran (TS 23%) during the trial storage period. For each size class, 12 trials were run, in which each factor combination was performed in triplicates. Our findings show that early instar larvae can be stored for a later use at 12.5 °C if they are prevented from drying out. Larvae weighing ≥1 mg have a higher rate of survival compared to larvae that weigh <1 mg. Survival rates of ≥1 mg larvae drop significantly after 7 days if no additional moisture is added. The addition of moistened wheat bran to prevent desiccation of the stored larvae increases the overall survival rate compared to adding no wheat bran or dry wheat bran. These findings can be a useful tool to store early instar larvae if not used within the early days after hatching.

Exploration of the effect of UV illumination on the production of house crickets (*Acheta domesticus*)

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House crickets have been identified as a promising source of food and feed due to their rich nutritional profile and their relatively easy rearing process. The aim of the study was to explore the possibility of introducing house crickets to an integrative system, where they can be grown together with algae and plants. UV illumination has been suggested to have a positive effect on the growth of various organisms. Thus, its effect on the life cycle and nutritional profile of the house crickets was evaluated. Crickets were exposed to UVB light (285 nm, 0.08 W/m²) for 4 h/day with an overall photoperiod of 8 h/day, until adulthood. A LED illumination system was used as a control illumination. Their individual weight (g/cricket), survival percent and growth performance were evaluated. After harvesting, an analysis of their composition was also performed. Results showed no effect of UV light on their growth performance or individual weight that ranged from 360 to 400 mg /cricket. However, the crickets exposed to UV (n=5) showed a 71±8% survival, while the ones deprived of UV light (n=5) showed a 62±4% survival after 53 days of rearing. Compositional analysis did not show any significant differences in fat, chitin, ash and total phenolic contents (dry weight basis) between the two different illumination systems. On the other hand, an analysis of variance of the data obtained by the two rearing treatments (n=5), with 3 analytical replicates per treatment (total n=15), showed that the crickets exposed to UV light had a significantly higher protein content (53 g/100 g d.w.) in comparison to the ones deprived from UV light (45 g/100 g d.w.). Therefore, exposure of house crickets to UV showed potential to be implemented in commercial integrated rearing systems.

Impacts of oviposition substrate availability on mating frequency and fertile egg production in the black soldier fly

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In industrial settings, BSF are reared to adulthood and housed within insect cages where they are presumed to mate. However, males are known to be aggressive and fertile egg production is highly variable, suggesting sexual conflict is negatively affecting success within the system. Oviposition is induced by placing within the same cage an 'oviposition box' that houses a substrate and conspecific larvae to attract females. Despite this method being widely accepted, we hypothesised this was a critical failure in design because it might lead to wasted reproductive effort (e.g. males often 'dogpile' onto females), whereas in the wild, females are able to disperse from these groups to locate an oviposition substrate. Therefore, to potentially reduce sexual conflict (in this case, male aggression) and serve as a proxy for the site-level differences that would be experienced by wild flies, we investigated the effect of introducing oviposition boxes 1-day after peak mating, as well as the start of each trial. The fully-crossed design (4 treatments, 3 replicates each) also included controls with boxes empty of any substrate. Preliminary investigations revealed that delaying the introduction of the oviposition substrate significantly increased total observed matings but had no effect on the frequency of male-male interactions, which decreased as flies aged. Observations also revealed that when substrate was absent, flies preferred to oviposit in locations other than cardboard traps, leading to a near total loss in egg collection, and suggesting vision is not the primary means by which BSF assess crevices for oviposition.

Insect farm design and good practices – making insect farming innovation accessible and scalable

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Insect farming has been proposed as an opportunity to positively transform the food system by introducing more circular nutrient flows and converting the up to 30% of food that is wasted globally into sustainable sources of nutrition. The black soldier fly has emerged as the insect of choice in the insect as feed space to address this opportunity. The field has seen tremendous progress in the last 3-4 years, supported by an ever-increasing body of academic research, positive regulatory developments and increasing market understanding of the products. More and more investments have been flowing into this nascent industry to facilitate the development of insect farming systems and technologies, led by the construction of large-scale, capital-intensive insect factory-farm production facilities. However, this large-scale factory-farm model might face limitations and risks in terms of economics, scaling potential, disease & supply chain risks, sustainability potential and accessibility of the technology. In this study, we present a detailed analysis of the insect value chain and different farming concepts from large-scale factory-farm to container-based farming units. We propose the existence of four discrete stages in the value chain and present an overview of the processes, material flows and metrics. Focusing on the insect farming (rearing/grow-out/bioconversion) part of the value chain, we further introduce a standardised, technology-enabled, data-driven light-weight insect farm facility blueprint and production model that aims to make insect innovation more accessible as a local utility-like model for renewable food and nutrition.

Data collection software for black soldier fly farming operations: empowering production teams with data

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Farming insects requires an immense amount of data collection. From environmental factors to lifecycle events, to moment-to-moment sampling of insects, there are too many data points we want to be able to easily capture them all. This data collection needs to be integrated into colony management as seamlessly as possible, allowing even small teams to gather data on the farm without interrupting their workflow. As a software engineer that's working on maggot farming problems, here are some key takeaways that I have observed that help make better software for collecting production data. The most critical component of developing this software is narrowing what data collection down to what the client needs. Our production team at Goterra has been heavily involved in the design of the software systems, providing clear information on what processes they need insight into for their operations. The software has been created to be agile enough to grow with Goterra as we expand our production operations to multiple global sites, focusing on collecting the data now to prepare us for in-depth research and analysis later. To do this, we created our own platform for collecting and analysing that data generated during Goterra's insect farming operations. The user experience was the focus of the application, since it is key that all members of the production team can easily access and enter data with minor interruptions to their daily tasks. We found that existing solutions were cumbersome and dense, with large tables of data to enter, but no connection to the work being performed on the production floor. At Goterra, we have created a solution that follows the production process, making data collection accessible to all team members, and providing automatic reporting to the team on the effectiveness of operations. The research explores the challenges faced with delivering a data analytics capability to a team required to perform production operations at scale. Continuous learning and learner experience design onsite help support applied research and experimental development carried out by Goterra. This relationship enables development of a robust solution when dealing with varying and contaminated organic wastes based on food and packaging trends.

A robust rearing crate system for black soldier fly production

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As the basic management unit in the black soldier fly larvae production system is the individual crate, the choice of the crate could have a decisive effect on the efficiency of the factory operation. Choosing the right crate will take consideration in many aspects, for example, the style, material, construction, size, and the potentials. However, there is very limited crate options specifically designed for black soldier production on the market for trading. Here SPR Agtech is presenting a crate system specifically designed for black soldier fly production with big potential to adapt into many more applications.

How to treat insects when they come into the world

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Someone has stated 'A new job is like a blank book and you are the author', likely a graduate engineer who comes fresh into the working world needs guidance, grooming and should know their interests. They listen to different opinions and they tend to choose the wrong path and it gets late to decide what they were actually meant to do. When I started my journey with Goterra as a Graduate Engineer, I listened to different opinions and was ready to take the path forward, which I was not confident in. I used to get stuck at each point with a '?' because I was not familiar with what I had to do? What were my responsibilities? What were my interests? I started listening to people about their ideas, their goals and got distracted, which led to a different path. Then comes my CEO into the picture, asking where you want to see yourself in the coming years? What do you want to become? Then I started thinking and stated I want to know strengths and weaknesses, and learn the designing process. She said 'Lets do it' and assigned me a small project which includes designing, leadership skills, project management skills, documentation and a lot more. Throughout the process, I got motivated and became confident about my goals, about the process, which kept me going and surviving into this working world. Likewise, when an insect comes into a world we need to decide the path and right direction and grow them accordingly. We should also know where we want to use them and start grooming them with proper maintenance like I received from my CEO. In Goterra we are doing the same thing, doing many kinds of research to use the different insects and utilise them so that they can be a better chance to the coming world and our process has begun. We are using black soldier fly for managing organic waste and giving all the care like a little baby from the initial stage:egg to the adultery. Much more is coming in our new projects, with different insects, and will make our world better and different.

The importance of implementing life cycle performance control points in a large-scale black soldier fly nursery

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During the approximately 45-day lifecycle of the black soldier fly (BSF), *Hermetia illucens*, the organism achieves three major transformations (from egg to larva, from larva to pupa and from pupa to imago). External factors such as the mode of operation, environmental conditions or quality and quantity of feed have an impact on how successful and efficient the BSF will pass from one life stage to the other in a nursery. It is thus important to be able to measure this impact on each life stage in an effort to improve the efficiency of rearing the BSF in a controlled environment. At the Food for the Future (F4F) BSF biowaste conversion plant in Maule, Chile, a nursery system was developed which captures the various life stages of the BSF and thus allows for a monitoring system to collect data on the exact number and moment of this transformation process. Neonates are collected and weighed again to measure emergence. Larvae of the second instar, ready to process biowaste, are weighed and counted to measure the survival rate between the first two instars. During the grow out, larval weight and quantity is measured at the 5th instar resulting in the survival rate between the 2nd and 5th instar. A selected group of larvae will continue to feed until reaching the pre-pupal stage. Their mass and quantity are measured again before the pre-pupae are portioned and placed in pupation units. The pupation units are placed in environments from where emerging flies can be quantified and dosed into cages resulting in the adult emergence rate. Lastly, the harvested amount of eggs from each cage are weighed and will define the number of eggs deposited per fly. The sum of all the implemented performance control points resulted in an overall required recycling rate of <1%. Looking in detail at each control point, the survival rate of hatching neonates and larvae up to the 2nd instar can be improved. The collection and interpretation of this data allows the nursery operator to recognise changes in the performance of the individual production steps in a timely manner and to react accordingly. Operating a monitoring system therefore pays off quickly, despite the increased effort in terms of personnel and infrastructure.

Production optimisation of edible insects in Québec: challenges and potential solutions

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Currently, in North America, nutritional entomology industries are under-developed, and the economic benefits needs remain to convince large-scale producers and consumers. In Québec, the current approaches for producing and transforming are focused on mealworms, crickets, and black soldier flies. Each are focused on specific life cycle characteristics, requirements for breeding and optimisation needs. Therefore, it is essential to: (1) Test the rearing conditions to optimise yields and nutritional diet optimisation; (2) Develop automated production processes to reduce production costs for mealworms and black soldier flies; (3) Validate optimisation in large scale production facility. The research teams have conducted several projects focusing on mealworms (*Tenebrio molitor*) and black soldier flies (*Hermetia illucens*). The preliminary results in optimising breeding culture and mass production were achieved through collaborative and retroactive feedback loops of multi-disciplinary approaches between entomology, engineering, and external partners. The optimising breeding culture projects have also highlighted the workforce and manutention time required as costly challenges. Therefore, technological transfers to any large-scale edible insect production and transformation, requires optimised automated processes at critical steps such as breeding, harvesting, and processing. The first automation project resulted in the development of a virtual modeled system to feed and sort mealworms, to perform as fast as 6 sec/tray and 30 sec/tray respectively, for a total of 27,000 trays/40 h. It also includes a procedure to retrieve the frass. The second automated system can attract black soldier flies thanks to a light source; a fine particle counter coupled to a one-way separation gate can isolate batches of 250 flies/min, prior to their transfer into the egg laying chambers. Both automated systems remain to be developed and optimised, as well as effective standardised operating procedures, but offer promising alternatives. The aim is to provide validated and technical solutions for production chains, without incurring additional excessive cost on in terms of equipment, inputs, and workforce.

Substrate physical properties as a driver for black soldier fly larvae growth rate

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The growth and nutritional profile of the BSFL larvae is usually investigated and compared when the larvae feed on substrates that differ in the chemical composition as well as physical properties. The current study aimed to neglect the effect of substrate nutritional composition and compare the larvae growth rates on substrates that differ only in the physical properties. The tested substrates differed mainly in their bulk density, water holding capacity, and rheology. In a factorial experiment, the substrates consisted of 74% water, 20 or 14% chicken feed (first factor: nutritional value), and a rest fibre (Second factor 'fibre component': powder cellulose with average fibre length of 700 µm, concentrated lignocellulose with an average length of 280 µm, or straw powder with less particle size than 2 mm). In a penetration test (Rheology) the substrates differed in the force-per-distance required and ranged from 0.019 to 0.457 N/mm despite the equal dry matter content of all substrates. Also, the different fibre components lead to different bulk densities of the substrates that ranged from 96 to 153 g/ml. The results show that the factor 'nutritional value' had the strongest impact on most larvae growth parameters. The substrate rheology values obtained did not correlate with larvae growth rate, but the bulk density of the substrate did. In the 'low nutritional value' treatments, substrate with high bulk density lead to faster growth rate and larvae reached their maximum growth in 6 days of instead of 7. Both tested factors (nutritional value, and fibre component) had an influence on larvae survival that ranged from 86 to 96%. Physical properties also influenced substrate drying, which ultimately influences the feed availability for (and the nutrient composition of) the larvae. These observations are important for the optimisation of BSF rearing substrates in order to shorten the growth rate without improving feed nutritional value when the management of wastes is a target.

Time to hatch a new colony maintenance plan? – age impacts reproductive output for *Hermetia illucens*

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A primary strategy for colony maintenance of the black soldier fly (BSF), *Hermetia illucens*, can include a continual release program in which newly-emerged adult BSF are introduced into a breeding-cage with older individuals from previous emergence events still present. The continual release strategy is blemished colony production, sometimes with remarkable peaks and valleys in both egg production and hatch rate, with little insight as to the driving factor(s) for these disparities. Results from the current study indicate age of soldier flies within a breeding cage can significantly impact the reproductive output of the colony. Three replicates of four treatments were repeated across two trials. Breeding cages consisted of an equal sex ratio (100 of each sex) of the following combinations: 2-d-old male (m) with 2-d-old female (f); 2-d-old m with 6-d-old f; 6-d-old m with 2-d-old f; 6-d-old m with 6-d-old f. Total number of matings observed over the course of the experiment was consistent across treatments. In general, egg production was consistent across treatments with young f and was not significantly different; however, variability in egg production was twice as large ($P < 0.05$) for treatments with old f than young f. Preliminary data also indicate hatch rate was driven by age of individuals; with young f and m resulting in 66% egg hatch, and all other trials including old individuals resulting in >5%. These data shed light on the challenge of high variability and unpredictability in fertile egg production that BSF breeders encounter. Removing old adults from cages is recommended as a means to reduce associated hatch variability.

Too hot, too cold: scaling effects and standardised research for industry relevance

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Harnessing the power of insects to feed the world is becoming an important and increasingly more pertinent aspect of achieving global food and feed security. In order to be viable for industry, an enormous quantity of insect biomass needs to be consistently produced on a daily basis. Multiple companies have developed large-scale production facilities (>10,000-m²) to meet the growing market demands for insect-based food and feeds. Research in this newly emerging biotechnology field, however, has mostly been limited to smaller scales, for both in-house R&D departments within the industry and for academic institutions. Trials conducted at the bench scale (rearing units <1 m²) can manipulate environmental conditions to precise and narrow ranges of temperature and humidity, which is important for achieving repeatable results and highlighting differences among experimental treatments. However small-scale results under tightly controlled conditions may produce results that are not applicable and/or relevant to the reality at industrial scales. In this presentation we review data collected from in-house trials on black soldier fly rearing that highlights the importance of scale and environmental parameters in impacting insect health and overall performance. We discuss how scale impacts the conditions insects are exposed to at multiple life stages, ultimately affecting final performance and overall plant productivity. Considering scale when undertaking academic and industry research will prove vital for advancement of the insect industry. Through continued partnership and collaboration, we hope to refine strategies that will enable research of the highest quality, and research that is extremely relevant to both industry and academia alike.

Scaling up production and processing equipment for yellow mealworm farming

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Small to medium scale insect farming is manually labour intensive, leading to high production costs, low efficiency and worker hazards. Medium to large farms generally conduct or outsource research and development to improve production practices, but smaller farmers do not have the same capacity. High production costs, lack of commercially available technological solutions or scientific expertise and unadapted governmental regulations represent obstacles to the expansion of the North American edible insect farming industry. The optimisation of production, notably through automation appears essential. Critical steps in yellow mealworm farming involve screening of the different life stage (adults, pupae and larvae) as well as separation of frass and unconsumed food particles. Uses of residuals organic waste from food processors to make insect farming economically viable also comes with its challenges. TriCycle, (Montreal, Canada) is a small-scale pilot farm of 1000 sq.ft, producing 3 tons of fresh larvae per year, and the data gathered over two years of operation confirms that feeding and sorting insects are the most time-consuming tasks. The screening process alone can represent up to 60% of farm operations, limiting upscaling of production. Meanwhile, this task is easily amenable to semi-automation. The sifters tailored for other industrial processes are often imperfect for insect farmers (grids unadapted to insect morphology and behaviour) and wet feed delivery in insect crates can be challenging (fruits and vegetables pulp). Custom equipment is generally quite pricey and requires time to be developed. Existing equipment for farm production can be used and integrated to insect farm to reduce repetitive labour at an affordable price for smaller insect producers. The current research surveyed available equipment to develop a mid-scale pilot plant for mealworms with semi automation. Constraints identified were addresses in the scale-up of the Montreal farm that will allow the production of 50 tons of fresh insects per year. Also, particular attention has been aimed at reducing worker exposure to dust, a significant human health hazard in insect productions.

Optimising insect rearing chain through the development of a model-driven decision support system

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Rapid growth in insect industry is projected during the forthcoming years. To meet the industrial scale in insect production, it is however necessary to enhance production efficiency, reduce labour intensity and markedly decrease the production cost. Cost structure in insect production is complex and the costs are affected by every day decision making in insect production facilities. To help insect farmers make economically sensible decisions, we develop user-friendly model-driven decision support system (DSS) for insect farmers by using a system design approach. In this presentation we will provide an outline of the DSS. The model uses data on insects' life cycle, production biology and performance on different substrates. Printed sensors, robots and an automated DSS will be integrated into the model. The model provides recommendations to repetitive management problems based on fixed action rules and workflows. DSS structure is developed in collaboration with end users. Finally, the DSS will be parametrised, tested, and validated with real cases. CoRoSect project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101016953.

Impact of container size and feed administration on *Hermetia illucens* rearing performance

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The increasing food and feed demand results in a greater need for alternative protein sources. Insect proteins have been identified as promising and sustainable alternatives due to their high feed conversion efficiency and biomass quality. These characteristics, plus the capacity of growing on a large variety of organic waste, make *Hermetia illucens* (Diptera: Stratiomyidae) one of the best candidates. Particularly, within the framework of circular economy, larvae of *H. illucens* are able to develop on organic fraction of municipal solid waste (OFMSW) which represent a valuable material (containing 16-28% of proteins and 11-29% of fats) and to convert it into high-value biomass (containing a very high percentage of protein 36-48% and fat 31-33%). As rearing condition can strongly impact *H. illucens* performance, the aim of the present study was to obtain important information, focusing on container size and feed ratio to optimise the larval production. Two container sizes were evaluated with a larval density of 0.38 larvae/cm³: the first one measured 60×40×11 cm and contained 10,000 larvae and 15,000 g of feed (OFMSW); the second one measured 21.5×14.5×4.2 cm and contained 500 larvae and 750 g of feed (OFMSW). The two feeding procedures that were evaluated consisted in providing the total amount of feed at the beginning of the trial or split in three times (day 1, 4, 8). Four replicates were set up for each trial and the larvae were collected when 5% reached the prepupal stage. ANOVA was used to test the effect of these factors and their interaction on different performance parameters: survival, final larval weight, final biomass, substrate reduction, efficiency of conversion index (ECI), waste reduction index (WRI). The results confirmed that larval number along with the associated container size and/or feed ratio are important factors to consider for *H. illucens* production. The higher final larval weight was obtained from larvae reared in bigger containers. Moreover, in such case, final biomass (DM), survival, ECI and WRI were significantly higher when feed was provided in one single solution.

Preference of *Hermetia illucens* oviposition sites in industrial breeding context

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Gravid black soldier flies are known to lay eggs in cracks and crevices near or on decaying organic matter. Commonly used attractants are based on decaying matter with or without larvae near to which is positioned an egg laying structure such as cardboard flutes, layered wood slats, and various plastic structures. In an industrialised and high density rearing situation, the tendency of females to lay their eggs in cramped, shady and humid places can lead to rogue egg laying and a marked decrease in productivity. It has been previously shown that the selection of the oviposition site in insects is induced by the presence of volatile compounds, exposure to light and the presence of hormones. Here we tested different attractants in order to differentiate between the attractiveness of feed and microbial community. We made 4 attractants available to females: Gainesville and, vermiculite (an inert substrate), both either with or without an inoculum of BSF community bacteria. We then collected the eggs laid over each substrate every day. Gravid females selected laying structures positioned over Gainesville food without preference for the inoculation status. These results promote the uses of simple attractants which can represent savings in costs and energy compared to a more complex attractant.

Optimising of the black soldier fly oviposition using new odour attractants and egg collectors

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The production of black soldier fly (BSF; *Hermetia illucens*), is rising worldwide due to the larval ability to convert organic wastes into a biomass rich in proteins and lipids that can be used for feed sector. The optimal mass production of BSF relies strongly on the stability of egg production and efficiency of the applied methods. To date, the egg trapping technique including an odour attractant and egg collector is a commonly applied method in BSF farming industry. One of the key steps of this technique is to apply the odour that attracts flies to lay eggs on a specific spot. The trapping efficiency of odours relies heavily on the type of volatile organic compounds they emit and their persistence. Decaying materials are known for their ability to attract the BSF females for oviposition. However, the composition of these materials is not stable over time and may contain pathogens that may affect the viability of the eggs and may pose a safety hazard to personnel. Therefore, there is an urgent need to determine a suitable, safe, and economically feasible odour that effectively controls the oviposition behaviour and subsequently ensures the efficiency of the BSFL production. In the FlyScent project we assessed a series of decaying materials using Proton Transfer Reaction- Mass Spectrometry (PTR-MS) to identify their key volatile organic compounds and subsequently used them to formulate synthetic attractants for BSF oviposition. In addition, we have developed new egg collectors ready to use for industry. This work shows the progress of the FlyScent project and presents the results from different tests on both egg collectors and odour attractants.

Dry processing of insects – how does it work in theory and praxis

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The insect industry or industrial insect sector is not new, but presently developing on a fast and high level. Current challenging tasks are the large-scale rearing and breeding which can be compared to a commercial animal rearing facility. In comparison to this the technology readiness level of the processing part is already high. Evolving market needs for pet food or aqua culture will lead to more customised processing concepts. Beside these theoretical design aspects of processing insects also several practical points enter the game, like finding a balance between price and quality. With a history of over 8 years in the insect industry REINARTZ developed numerous dry processing solutions for black soldier flies, mealworms and crickets and implemented up to 40 in field installations. Based on the insect farmer's figures of wet larvae capacity, harvesting strategy and shift plans the dry process will be designed. Evaluation of the drying technology is one key point of a sustainable and successful insect processing. The other essential topic is that separation with screw presses achieves traditionally high defatting values and leads to high protein rates. Typical results are around 8% of residual fat and a protein rate exceeding 60%. In order to meet market demands in regard to digestibility and solubility the process temperatures can be precisely maintained. The trend towards decentralised rearing and processing facilities leads to small specialised units, flexible but focused on high quality products. Therefore, the dry processing is the blueprint for a successful business case.

What breaks the temperature size rule – maggot heat matters

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The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae) is being used for bio-waste recycle agents and novel feed resources globally due to its economic value. We measured larval growth performance at different population size (e.g. 10,000, 1000 and 166 larvae with the same larvae to feed ratio) and temperature (e.g. 20 and 30 °C) to simulate different scales of production. Results indicate a clear interaction effect between population size and temperature. Larvae at low temperature do not always grow bigger but results were density dependent. Considering other variables measured (e.g. duration, maximum substrate temperature, feed conversion ratio, feed digestibility, and digested feed conversion efficiency), maggot heat generation seems to be an important clue revealing strategies used in balancing feed acquisition and energy allocation. Larvae with small population size at low temperature did not generate enough heat to digest the diet effectively, which resulting in the observed temperature size rule violation phenomenon.

Impact of starving time on the fitness of black soldier fly (Diptera: Stratiomyidae) neonates

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The mass production of black soldier fly larvae (BSFL), *Hermetia illucens* L. (Diptera: Stratiomyidae), for use as a composting agent, waste management and alternative protein source for animal feed has gained attention in different regions of the globe. Within the production cycle, the fattening stage of the larvae is the one that most demands food and draws the attention of the producers of this insect, since the last larval instar, the so-called prepupae, is the final product with the highest commercial value. However, the success of this stage is extremely related to the fitness of the neonates that will be part of the fattening process. Newly hatched larvae (neonates) represent the most sensitive phase of the BSF biological cycle, and require great efforts to understand, within the management, how much the lack of food at the right time can interfere with its development and performance. Therefore, this study aimed to evaluate the biological characteristics (final weight and viability) of neonates in different starving periods. The experiment consisted of 3 treatments and 10 repetitions, as follows: (1) <24 hours (control); (2) <48 hours and (3) <72 hours. The effect of starving time on the viability of neonates was analysed by fitting a linearly generalised model, assuming a binomial error, and the means were compared using the Tukey test. The weight of neonates was analysed assuming normal distribution (ANOVA). The results found in this study indicated that the weight and viability of neonates starved for 24 and 48 hours do not differ, but both differ significantly ($P < 0.05$) from those starved for 72 hours. Understanding how stress due to food and nutrient restriction biologically interferes with this insect is essential to meet the daily needs of its mass production, since it can change the newborn collection routine or even interfere with the estimation of viable newborns that will be transferred to the fattening stage, and thus contribute to the successful establishment of new production systems.

Harnessing integrated multitrophic ecological services for food production in deep space and remote regions on Earth

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In January 2021, the Deep Space Food Challenge was launched by the Canadian Space Agency. The goal of this competition was to develop new technologies to provide quality food for astronauts on long-duration missions, while minimising the resources required for their production. To reach this objective, we designed an advanced food production system used to transform the crew's residual organic matter into a variety of foods (fruits, vegetables, spirulina, eggs and meat). Our technology ecosystem includes an automated black soldier fly larvae rearing system to bioconvert organic residues into quality feed ingredients for quail production of eggs and ultimately meat at the end of their laying cycle. Our proposal also includes photobioreactors generating fresh spirulina (edible cyanobacteria) as well as a horticultural production wheel in which fertilised plants are grown from the larvae frass. Specifically, the fly production system consists of a wheel, at the centre of which a production module produces larvae every day that are automatically transferred to one of 11 production boxes fixed around the circumference of the wheel. Boxes are indexed to a new position daily. Humidity and ventilation are controlled inside the production box and the module in closed circuit. Larvae and frass are harvested at day 11, after inserting the box in the microwave for a pasteurisation step that reduces bacterial load and kills the larvae. A compartment in the production box enables the easy recuperation of pre-pupa to continue the production cycle. Dead larvae and frass are separated by opening a grate inside the box and shaking. Frass is then transferred to the horticultural production wheel and larvae to quails as feed. The system aims to harness the integrated multitrophic ecological services of insects to provide astronauts with a diversified diet with minimal inputs. Earth-based applications could also result from our proposal to improve food autonomy and security of populations in remote areas. We present this innovative and sustainable project through a 3D video animation.

Harvesting & traditional role of eating insects/ sustainable consumption & food security

Consumer acceptance in insect-based food and feed in Europe – a choice experiment

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Insects are a promising alternative source of protein and micronutrients. Their use in human diets and as feed for livestock were included as strategies to support the realisation of the SDGs of food security, improved nutrition and responsible production and consumption. Yet, in Europe, low consumer acceptance for insects as part of a regular diet impedes their use as food. Strategies such as integrating insect-fed meat and priming nutritional and sustainability aspects of insect-based food can raise acceptance for insects as food and feed. There is evidence about consumer preferences for insect-fed fish but preferences for insect-fed chicken need to be investigated. Modifying the choice context was shown to affect food choice. It is therefore anticipated that priming consumers with the insect-protein content or the reduced climate impact of alternative meat products will affect their choice. In this study, we tested the consumer acceptance for insect-based and insect-fed chicken meatballs in Germany, Italy and Portugal via an online discrete choice experiment and survey (n=1,500). We examined the product attributes and personal characteristics, that influenced consumer preferences for insect-based food products and food products made of insect-fed chicken meat. We also evaluated their WTP. To inspect the priming effect, we included three conditions that vary the instructions framing the choice tasks. This study estimated the utility associated with the attributes and attribute levels and modelled the effect of trust and priming on preferences. Models were specified for each country using a mixed logit model to estimate the main attributes effects and a hybrid mixed logit model to include the main and interaction effects of priming treatment covariates.

Beetle grubs as a source of food and feed in western Kenya: cultural practices and nutritional status

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Although 31% of insect species consumed worldwide are beetles, particularly their larvae (grubs) that live on decomposing organic waste, there's limited research attention on their use as a source of food and feed. This study assessed socio-cultural practices on the use of grubs collected from farmyard manure in Busia, Bungoma, Trans Nzoia and Kakamega counties in western Kenya; and determined the proximate, mineral and flavonoid contents of the dominant edible grub species (*Oryctes* sp.). Exactly 211 households were interviewed alongside key informant focus group discussions. We found that the grubs were consumed and fed to animals (mainly pigs and poultry) in 39 and 78% of the households, respectively. The key benefits of the grubs were reportedly: (1) nutritious and delicious; (2) able to enhance animal weight gain and increase poultry egg numbers; (3) helpful in recycling nutrients from organic waste, thereby mitigating environmental pollution; and (4) not linked to any allergic reaction when consumed by humans. Toasting and roasting were the dominant methods of preparing the grubs for food. Exactly 66% of the respondents were willing to farm the grubs, provided that efficient rearing protocols and market for the grubs were available. About 98% of the respondents lacked knowledge of grub biology. The use of the grubs varied across counties and by gender, marital status, education level and economic activity of respondents. Lack of knowledge on the nutritional benefits of the grubs and stigma were key deterrents to other potential consumers. Crude protein, carbohydrate, crude fat and ash contents of *Oryctes* sp. grubs were 40-46, 13-23, 10-11, 1-2 and 1%, respectively. The grubs were also rich in minerals (e.g. iron, zinc and calcium) and flavonoids. Our findings demonstrate that the nutrient-rich grubs are an important source of food and feed in western Kenya. However, further research on techniques for their sustainable mass production and awareness creation on their contribution to food and feed security are needed.

Entomophagy in Myanmar: factors affecting consumption of edible insects as food

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As the population grows, so does the importance of nutrition. People have become more interested in eating a substitute for meat protein, to say exactly in this case, insects. Due to this interest, research is being done on insect consumption in the West, but research in Asia, particularly in Myanmar, is scarce. Although insect consumption is common in Myanmar, not everyone does. This research aims to investigate the acceptance of consumers and consumption frequency of edible insects and explore edible insects' potential contribution to malnutrition and food security by using descriptive and logistic regression analysis. Results show that 99% of the respondents are familiar with edible insects; 73% of the respondents, 38% male, and 35% female, have experience with insect-eating, about 67% of the respondents are currently consuming insects. The most preferred varieties are crickets, bees, and bamboo worms, followed by dung beetles, water scavenger beetles, and palm weevil larvae. The binary logistic results showed that gender, ethnicity, attitude, insect phobia, disgust, availability of edible insects, and availability of fish and meat have significant effects on consumer acceptance. The ordered logistic result revealed that gender, monthly income, ethnicity, food safety, naturalness, and taste significantly impact the frequency of consumption. Insect phobia and disgust are the most significant impediments to the development of the insect industry. Introducing the invisible form of the new insect products and providing information on producing or harvesting the insects systematically can help overcome this problem. The consumption frequency of wild insects is impeded by their seasonal availability. This highlights the need for more information on insect rearing and the benefits of consuming reared insects. Circulating that information via social media might increase the number of new insect consumers and the consumption frequency of current customers.

Perspective for edible caterpillar consumption in R.D. Congo

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Previous studies have shown the decline of many edible caterpillars in African countries. To reduce this threat, our research group in DRC aims to: (1) study the distribution of caterpillars and their host plants; (2) identify potential species for domestication; and (3) study harvesting and consumption patterns in different part of the country. To date, 146 species of edible Lepidoptera have been inventoried in Africa and 63 of them were mentioned in two field surveys performed in Masi-Manimba territory. Caterpillars are principally harvested during the rainy season and detected in forests (46%), savannah (19%) or the two (35%). Respondents confirmed a decline in caterpillar availability as only 22% of regular species seems to be easily available in the environment. Concerning preferred species, almost all respondents recognised the high taste value of *Imbrasia epimethea* and *Cirina forda*, which is widely consumed in the DRC and other sub-Saharan African. To ensure a long-term access to these natural resources for local population, it is important to provide a legal framework for the insect harvesting and management of local forests. Another alternative would be to set up breeding of edible caterpillar, but these two species do not show biological qualities facilitating their rearing. Although *I. epimethea* is found almost all year long in this territory, its development time is particularly long while *C. forda* is present only for two months and harbour a long pupation time. Among identified species in Masi-Manimba, *Aegocera rectilinea*, a species poorly documented as edible seems particularly interesting as our team already reared this insect on *Boerhavia diffusa* leaves under laboratory condition. Multivoltine and polyphagous, this noctuid is a suitable species for mass rearing and is already reared directly in the field in the Kwilu province. Nevertheless, before promoting this species at the country level, studies on consumer acceptability will be needed as our surveys in Kinshasa and Masi-Manimba indicate that edible insect preferences differ by region although certain species such as *C. forda*, *I. oyemensis*, *I. epimethea* are common to the whole country.

Entomophagy in western Kenya: the role of psychological and cultural factors

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This study utilises data from a random sample of 442 households from Bungoma and Trans-Nzoia counties in Kenya to assess awareness and determinants of edible insect-eating (entomophagy) behaviour. We also determine household preference for insects over meat. The study uses the extended theory of planned behaviour and structural equation modelling to determine entomophagy. Results indicate that attitude, perceived behavioural control, and subjective norms positively and significantly influenced households' intention to eat insects, while environmental concerns negatively affected intention to eat insects. Intention to consume is positively associated with insect-eating behaviour. Regarding household characteristics, we find the main factors explaining entomophagy, and differences in what insect is eaten, are cultural. Objective knowledge about insects is a strong predictor of preference for insects over beef – suggesting the positive role of nutrition information. These results show the importance of both psychological variables and culture in influencing entomophagy.

Edible insects in sensory-based and co-creative food systems pedagogy

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How can we use the topic of edible insects to engage diverse audiences in broader discussions about the agro-industrial food system? Edible insects offer a useful tool into critical conversations about the food system's environmental impact, social inequities, and colonial histories. Drawing from anthropological fieldwork behind edible insect educational booths, as an event coordinator with Little Herds, and as a university food systems instructor, this presentation will discuss the importance of sensory-based and co-creative food system learning for diverse public and student audiences. For entomophagy advocates and food systems educators, the topic provides important considerations for how we may best prompt publics to rethink food norms toward a more sustainable, equitable, and resilient food system.

Indigenous knowledge on medicinal plants to identify insect herbivores with beneficial bioactive activity

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Kawakawa (KK) (*Macropiper excelsum*), an endemic plant of Aotearoa-New Zealand (A-NZ) is a Rongoā rākau, medicinal plant, and is culturally significant to indigenous Māori. A recent survey seeking Māori knowledge on the cultural significance of native plants and insects, found that 95% of people identified KK as a rongoā rakau. Further, 80% of these individuals use or have used KK as a remedy. KK is used as an ointment, tea or for detoxification. Phytochemical studies have shown the presence of bioactives with anti-diabetic properties in KK leaves. The KK looper/whangawhanga (*Cleora scriptaria*) creates the characteristic holes in KK leaves and indigenous knowledge has identified that leaves eaten by the caterpillars have greater KK healing properties. Rapid evaporative ionisation mass spectrometry (REIMS) is a technique that allows fingerprinting and metabolomic characterisation of a sample without the need of sample preparation. This technique provides a method to easily identify differences in metabolites within and between KK leaves. KK looper were collected in and reared in cages on KK plants in Canterbury, A-NZ. KK leaves with and without caterpillar damage were harvested from KK plants and stored at -80 °C adhering to Māori principals. The REIMS laser technique was used to obtain volatiles from caterpillar eaten and non-caterpillar eaten sections from the same leaf and other non-eaten leaves from the same plant. It was hypothesised that sections surrounding KK looper damage would have a different metabolomic fingerprint to intact leaves and the non-eaten parts of the same leaves which would corroborate the indigenous knowledge. Differences were found in the metabolomic fingerprint of KK leaves supporting indigenous knowledge that the caterpillar causes a change in plant chemical composition potentially increasing the plants medicinal value. The KK looper should also be investigated as it could potentially assimilate compounds from the plant containing medical properties. This study forms the basis for research to ascertain the benefit from an endemic A-NZ species to provide beneficial bioactive with human health potential.

Manual harvesting of desert locusts as a scalable farmer-first nature-based response promoting food security

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If it had not been for COVID-19, the desert locust (DL) infestation in the horn of Africa from 2019-2022 would have been the greatest humanitarian crisis in 2020. For the 46% of Kenyans who earn less than \$1 per day, any resulting crop or income loss is beyond devastating. As a result, 35 million people have suffered food insecurity due to the recent infestation across the region. A community centred, farmer-first and nature based response to the DL infestation: Over a 6 week period in 2021, we created two mobile teams to hunt DL in northern Kenya. Through these teams, unsprayed swarms were targeted and communities mobilised and trained in locust harvesting techniques. Harvesters were paid 50 ksh per kg of live locusts harvested, thereby reducing swarm size while directly benefiting affected communities: Over 4,3 tons of live DL were harvested with Ksh 198,000 (\$ 1,835) paid to 180 affected people/households. We created techniques to convert harvested locusts into protein rich locust meal for animal feed, or nitrogen rich organic compost. Our nature-based learnings have been digitised and training has been piloted with hundreds of farmers. We would like to present how reimagining traditional responses to insect pests can create sustainable and holistic solutions that compliment government activities and promote food security in affected areas. Coupled with advanced weather and pest alerts, we can prepare government and communities in advance of a pest invasion allowing them to turn climate challenge into opportunity.

COVID-19 and entomophagy in DRC: contribution of edible insects to a more resilient local food system

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Restrictive measures against the COVID-19 are reported to threaten food systems. To mitigate their impacts, strengthening local food production is paramount. As entomophagy is an old tradition in Africa, insects should be considered as they encompass multiple dimensions of sustainability that could benefit to cities in building resilience. To that end, this study documents insects' consumption patterns since the onset of the pandemic in Kinshasa and the impacts of restrictive measures for consumers with different sociodemographic features. Data were collected through a semi-structured questionnaire (to 270 respondents) and Chi-square (χ^2) tests, analysis of variance and principal components analysis (PCA) were performed. Results indicated integration of entomophagy (77.7%) and no association with sociodemographic features, suggesting that insects are not a fallback food. Impacts of the lockdown and borders closure included price increase, a less accessible offer or less street vendors. A significant higher percentage of urbanites (than peri-urban or rural dwellers) reported to eat more insects compared to period prior to the pandemic ($\chi^2=8.283$, $P=0.016$, $df=2$) while the percentage of respondents reporting disruption was not significantly different between areas. Moreover, analysis of consumption patterns indicated the top 4 foodstuffs in terms of consumption frequency (these are fish, poultry, insects and beef) while the PCA suggested 3 groups with inequalities based on the frequency and total expenditure for insects and meat consumption. Considering restrictive measures in Kinshasa, current results endorsed the contribution of local food in building resilient local food systems thanks to short supply chain. As we look beyond the pandemic, efficient strategies that reduce inequalities and build resilience for local food system in DRC could be achieved by ensuring production of local food such as edible insects given that they are abundant (121 insects recently reviewed at species), cheaper than meat and at hand.

Inventory reveals wide biodiversity of edible insects in the Eastern Democratic Republic of Congo

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With its wide edible insects' biodiversity, the Democratic Republic of Congo is one of the most important biodiversity hotspots in Africa. This study aimed at giving a first insight on inventory showing diversity, perception, consumption, availability, host plants, harvesting techniques and processing techniques of edible insects in South-Kivu, DRC. It recorded twenty-three edible insects belonging to nine families and five orders, some of which are consumed in the larval, adult, egg and pupa stages. *Rhynchophorus phoenicis*, *Alphitobius diaperinus*, *Macrotermes subhyalinus* and *Acheta domesticus* were the most preferred edible insects in Fizi Territory, *Ruspolia differens* and *Apis mellifera* larvae in Kabare Territory, *Imbrasia oyemensis*, *Imbrasia epimethea*, *Rhynchophorus ferrugineus* and *Rhynchophorus phoenicis* in Mwenga Territory, *Ruspolia differens*, *M. subhyalinus*, *Gryllotalpa africana*, Nsike, *Nomadacris septemfasciata* and *A. mellifera* larvae in Walungu Territory. *Ruspolia differens*, *I. oyemensis*, *A. mellifera* larvae, *G. africana* and Nsike, were preferred for their taste. *Acheta domesticus*, *A. diaperinus* and *A. mellifera* larvae were abundant throughout the year, while others were only available for 9 months or less per year. Numerous plants have been recorded as their hosts, including plants used for food and income. Harvesting strategies and period, processing methods and preservation techniques depend on insect species, local knowledge and practices. These findings suggest similar and thorough studies on entomophagy across the country while encouraging the rearing of edible insects to address the existing high demand and environmental concerns.

Caterpillars consumed in Masi-Manimba territory (Kwilu), Democratic Republic of the Congo

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Caterpillars are a traditional favourite food that potentially represents the largest proportion of total animal protein consumed by people in Masi-Manimba, one of the territories in Kwilu Province, Democratic Republic of Congo. However, caterpillars are becoming increasingly scarce, with no inventory data existing for this region. This study aimed to contribute to the valorisation of edible caterpillars in Masi-Manimba through their inventory and identification. A questionnaire was completed by 212 people representing the four main ethnic groups in the territory and seven peripheral sectors. The caterpillars were collected in 2020 and 2021 in the township. Eighty-two species were listed by the four main ethnic groups, of which 63 species were scientifically confirmed. The main family was Saturniidae, followed by Notodontidae and Sphingidae. The genus *Imbrasia* is the most represented in the territory. Overall, 46% of caterpillars are detected in forests, 19% in savannah, and 35% across multiple habitats. Surveyed people considered 64% of caterpillars to be very tasty. Large numbers were harvested during the rainy season (November and January). Respondents observed that there has been a major decline in caterpillars, with just 22% of species being readily available. Due to this major decline in caterpillars, it is urgent to study their biology and relationship with food plants, to farm them successfully.

Survey of edible insects, with reference to harvesting methods, practices and consumption technology in Benin

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In a context of rising prices for conventional animal proteins, food insecurity, environmental pressures and population growth, the consumption of insects, or entomophagy, presents itself as an opportunity for the future. As an emerging practice, not yet fully integrated into the culture, supporting entomophagy requires a concerted effort to bring about sustainable societal change. Sustainable diets are diets with low environmental impact that contribute to food and nutrition security and healthy lives for present and future generations. Although insect's consumption in Africa is an ancient practice, little information exists regarding their diversity, availability, and consumption mode. Therefore, in order to remove the obstacles to the development of the edible insect production sector and to meet current and future food challenges, it is necessary to set up and coordinate research aimed at all stakeholders in the food landscape (consumers, etc.). The present study aims to inventory the geographic distribution of edible insects and entomophagy practices across the ten phytogeographic zones (PGZ) of Benin. A large socioeconomic survey was conducted in one municipality in each PGZ. Four hundred and twenty-two participants were enrolled through 40 focus groups and an additional 845 participants through direct interviews. Information on edible insects, method of harvesting, availability and frequency of consumption was documented in each PGZ. Frequency citation, frequency consumption and availability were computed and used to determine the most consumed insect species and to understand the main factors of acceptability (or rejection) and perception of entomophagy (cultural and organoleptic preferences by consumers) in Benin.

Pesticide residues were not found in the Mexican Chapulin (*Sphenarium purpurascens*) harvested from cornfields

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Pesticide residues were not found in the Mexican Chapulin (*Sphenarium purpurascens*) harvested from cornfields. Agricultural pest insects are consumed as part of the diet in many tropical countries. Chapulin a devastating pest insect common in central Mexico, damaging the cornfields. Traditionally, mechanical harvesting has been used for the survival of farmers to collect the insect as a source of food. However, in the last decades, farmers have increased the use of chemical plague control to reduce the economic losses produced by this plague. On the other hand, Chapulin is still collected from cornfields, mainly for survival farmers in lands where chemical pesticides could be used. Naturally, then, there is a concern about the presence of pesticides residues in Chapulin. So, the present research aimed to determine organohalides, organophosphates, methyl carbamates, organonitrogens, and other compounds in Chapulin harvested from cornfields. In accordance with current legislation and considering that there is no precise information on the type of pesticides used in the region, a complete analysis of pesticides was carried out. A total of 300 pesticides were analysed by AOAC 2007.01 (pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulphate), in a 3.5274 oz sample. The insects were collected in the region of Tepapayeca, Puebla, Mexico (18°40'15" N, 98°31'52" W). In this region, Chapulin harvesting represents 1.6% of GDP due to economic activity and its inhabitants are traditional consumers. Results showed no residue of pesticides detected in any case. Based on the lack of detection observed in the product, it could be considered that Chapulin is safe for human consumption. To our knowledge, this is the first report on pesticide residues in Chapulin. However, this is a limited study; more samples, metals, and microbiological analysis should be done to guarantee the safety of the final consumer.

Distribution and evaluation of the nutritional potential of edible insects in Djerem (Adamaoua-Cameroon)

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In the districts of Djerem in Cameroon, we identified the host plants of several edible insect species, the areas where there are collected, periods of the year when they were available, and insects' nutritional value. The survey was conducted by direct interviews. The host plant *Albizia zygia* was colonised by *Anaphe panda* and *Imbrasia obscura*, *Entada africana* by *Antheua* sp. and *Imbrasia obscura*, and *Mucuna bennettii* by *Cirina forda*. These caterpillars appear on their host plant between the months of June and September. Termites appear from February to May. Beetle larvae appear late September and October and between November and January. These insects are collected all over the Djerem. The chemical values of *Antheua* sp., *A. panda*, and *C. forda* revealed a highly variable nutrient content. The nutritional values of these caterpillars can solve the needs of proteins and energy deficiency among poor populations.

Perception, marketing & economics

Does scale really matter – modelling different black soldier fly facility operations

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In urban centres of low and middle income countries, treatment of biowaste remains an untapped approach given its perceived limited value. The black soldier fly larvae (BSFL) conversion process has however proven to be promising, given its potential to generate high value products with low technological input. Nevertheless, the question on how to achieve financial viability and how to integrate it into a given solid waste management system remains a gap in research. Data from a manually operated BSFL facility (2 tons waste/day) in Sidoarjo, Indonesia was used as input for a cost-revenue model. It is structured by differentiating each processing step of the BSF facility and assigning tasks, resources and respective costs by Activity Based Costing. Unit costs for salaries, energy or infrastructure can be individually adjusted depending on the local context which then allows the calculation of material and operating costs at different scales as well as common key financial indicators. The study modelled different scenarios on how the processing units of the BSF facility could be disaggregated in separate business units with varying degrees of decentralisation. One example is to operate a central nursery while decentralising many small BSFL waste treatment and post-processing units. Results show that such a scenario shows both commercial and environmental benefits. Treating waste close to the source of waste generation reduces transport, creates local jobs and enables the integration into the existing waste management system of local governments which already operate decentralised waste treatment/recycling stations. Such waste management, or in other words ‘decentralised insect farming’, is comparable to existing livestock farming activities, be it aquaculture or poultry farming, where small and medium-sized farms contribute significantly to the food security of the region and create jobs. The analysis of the different scales and cooperation models shows that savings through ‘economies of scale’ are not necessarily the only determining factor when envisaging BSF biowaste processing as an integral part of the solid waste management system

Analysis of costs and revenues of insect farms in the Netherlands

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In the past decades, a great deal of research has been done into the production of insects in various fields (e.g. optimising insect growth), but empirical research into the economic viability of insect farms in Europe is limited. An understanding of the profitability of insect farms is crucial, not only for rearers to determine their position in the market, but also for the starting entrepreneurs prior to the decision to start. In addition, it is needed for investors, insurance companies and creditors to have insights into the viability of the industry to make an informed decision in providing services. In this study, we analysed the costs and revenues for *T. molitor* farms in the Netherlands, distinguishing between the business models reproduction (n=2) and rearing (n=5). In addition, using expert elicitation (n=5) we obtained estimates on current sales prices, as well as feed and investments costs for *H. illucens* farms (reproduction and rearing integrated). Revenues for *T. molitor* farms included those obtained from fresh and first-processed (dried) products, insect frass and other extension services. Regarding costs, fixed and variable costs were investigated, as well as investments. Preliminary results from stochastic simulation using the obtained data showed net present value (NPV) and the return on investment (ROI). Results of sensitivity analysis based on spearman rank correlation coefficient between NPV as well as ROI and operating profit showed that the main determining factors for profitability of *T. molitor* rearing and reproduction farms included labour and substrate costs, and sales prices. Results for *H. illucens* production highlighted that main costs components included labour, feed and depreciation costs. This study provides first estimates that can be used in decision making on (credit) risk assessment for service providers and designing targeted interventions by policy makers. In addition, the results are a basis for further research into the most optimal and (cost) efficient form of insect production.

Promoting insects for food and feed in Myanmar: TV cooking shows and social media promotions

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Eating insects has been popular in Myanmar throughout the ages. To understand and document the sector many locations and topics have been studied. Market stimulation has been a key request from insect farmers. Studies have shown that there is a strong interest to eat insects as snacks (85%), but less as main meals (45%). There are two strategies to change this are: (1) main meal recipe creation, via the 'Everyday Insects' recipe book; (2) broader social media promotions. With images of delicious local recipes and meals available from the recipe book, the idea of a TV cooking program emerged. Initially a 'Master Chef' style program was considered. However, combining guest chefs, visits to local farms, multiple kitchens and travel in rural Myanmar was favoured more. To date 2 series of 9 episodes total, each between 10 and 20 minutes were produced in the MITV 'Let's Taste Delicious Insects' series. Social media growth has been rapid in Myanmar since telecoms sector deregulation from 2013 allowed rapid internet penetration. In January 2021 there were 29 million social media users (53% of the total population), with a social media usage growth of 32% per annum, Facebook being the leader with 27 million users. A specific 'Everyday Insects' FB TV channel followed book release, with host chef engagement, short cooking videos, and news articles on the global insect industry. Brief cooking video clips were filmed to fit with short Facebook viewing spans. Peak episode engagement has exceeded 430,000, with cooking videos being the most popular. Ongoing programs for increased exposure are planned. Food promotions were coupled with blog posts on industry news and farming knowledge. Myanmar translations of international documents have proven extremely popular. For example, the ICIPE guide on black soldier fly production has had 1,884 Myanmar and 1,308 English downloads. The recent World Bank 'Insect and Hydroponic Farming in Africa' has had 309 downloads which makes Myanmar the second highest downloader (12 Feb. 2022). The promotion strategies used have worked well to stimulate an already receptive market. Needed now is more investment in farming and processing to assist interested insect farmers.

Introducing edible insects into animal feed in Cameroon – a new opportunity?

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The population of Cameroon triples every thirty years, which is increasing the demand for proteins. Edible insects represent acceptable food for fish and livestock that naturally feed on insects. Unsurprisingly, there are numerous examples of feed formulations based on whole raw or dried insects. The objective of this study was to determine what place insects could take as a natural and sustainable source of protein in aquaculture and livestock feeds in Cameroon. To elucidate this question we made survey of the animal production sector, with an emphasis on aquaculture, poultry farming and pig farming. Then we analysed the potential of edible insects and the opportunities for their valorisation in the animal production sector in Cameroon. The socioeconomic and technical data collected from April to August 2018 from 32 feed mills and 72 breeders (aquaculturists, poultry pig farmers) in central Cameroon. The main feedstuffs used are corn, soybean meal, peanut meal, concentrate and fishmeal. The cost of these different feedstuffs changes according to the growing seasons, availability on the local market, and quality. The perception of breeders and feed producers about the use of insects in animal feed is mixed; breeders are more enthusiastic than feed producers about the idea of integrating insect-based meal into feed compositions. However, the convergence of all the players involved in the value chain should ensure the good quality of the finished product, its societal acceptance and the technical and economic performance of the farms, but the crucial question will remain the price of the final products and their availability all along the year.

Visual history of ideas about insect-related consumables: the case of *Dactylopius coccus* and *Ophiocordyceps sinensis*

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Central to this paper is a discussion of the role of images and labels historically (1520-2020) in the promotion of consumables structurally derived directly or indirectly from insects. The main focus lies on two study cases of visual marketing strategies. The first one involves the industrial use of *Dactylopius coccus* (cochineal scale insects), first as a dye and eventually, as colour additive in food. Popularly known as carmine, E120, as it is registered in EU food control documents, may be found, under certain regulations, in fruit-flavoured breakfast cereals, sausages, pâtés, drinks, cakes and milk products. The second case considers the role of images and labels in the circulation of an entomopathogenic fungus, *Ophiocordyceps sinensis* (caterpillar fungus), as a popular addition in the landscape of health supplements and health-enhancing consumables. The main hypotheses are: (1) edible-non-edible hierarchies of food sources become visually more prominent through images and labels that have no didactic purpose, whereas informative pictorial representations challenge fixed preconceptions about edibility; (2) preconceptions about health are underlined implicitly in images and texts and used as a reference to validate the edibility of consumables directly or indirectly related to insects. Methodologically, visual data, visual research, discourse analysis, close reading and text data mining (N-grams) are applied to a corpus of 300 images and texts, dating from the 16th century to 2020, and selected based on their retrievability across digital platforms and attention received within public discourse. As this paper shows, images have tacitly registered carmine's journey from Mesoamerican indigenous settings to ice creams advertisement posters in Western Europe, obviating the actual nature of the substance. In stark contrast with carmine, the insect-related origin of caterpillar fungus is by no means hidden in the construction of images and labels used for advertisement purposes. The main conclusion is that pictorial representations and linguistic classificatory protocols provide insights about the diffusion and circulation of ideas and preconceptions about insects, not necessarily as food, but either as healthy or unhealthy food sources.

The effect of labelling and visual properties on the acceptance of foods containing insects

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Introducing insects as a source of nutrients (e.g. protein) plays a key role in many countries' environmental policies. However, westerners generally reject insects as an ingredient of food products and meals. The aim of our study was to assess if explicitly labelling food as containing insects and/or implying it by manipulating the appearance of food influences the participants' perception of food products or their behavioural reaction to such products. The participants were asked to try a range of foods none of which contained ingredients derived from insects. However, the experimental conditions varied with regard to food labelling (insects content) and appearance (traces of insects-like ingredients). We observed the participants' non-verbal behavioural reactions to the foods. Then, the respondents filled in a questionnaire evaluating food properties. Additionally, we asked the participants to fill in a set of questionnaires measuring other variables (food neophobia, disgust, variety seeking, etc.). The results showed that products labelled as containing insects are consumed with reluctance and in lower quantity despite their appearance. Recent online study confirmed the results that the presence of an insect content label has a negative effect on the evaluation of the product. The effect was greater when an insect content label was combined with an actual image of an insect.

Packaging communication as a tool to reduce disgust at edible insect

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Insect based snacks, protein bars, mueslis or pasta have been on the European market for a few years, however, they are still far from being successful. Disgust at eating insects has been identified as a primary barrier to consumption. Amongst the strategies to overcome this hurdle, the presentation of informative or visual elements has shown to be effective. The product packaging as being referred to as the silent seller bears the potential for presenting elements for the reduction of disgust. This study investigates the potential of packaging communication on reducing the disgust of insect-based foods in Germany. An online survey was conducted using chocolate bars with insect powder as a model. The four hundred twenty-two participants were confronted with ten packaging designs representing different visual and informative elements such as labels and claims. The tested elements were shown on a rather neutral test packaging in order to be able to compare the individual effect. The results showed that images of ingredients, such as chocolate and hazelnuts, on packaging are particularly effective in reducing disgust. The same applies to a transparent window in the packaging. The presence of the organic label and the new Entotrust label significantly increased the assumed food safety. Statements about protein content and sustainability were less effective, and the image of a cricket had a significant impact on increasing disgust. Our results suggest that the packaging design should be used consciously to reduce disgust and thus support the purchase probability.

Development of a lexicon and sensory profiles for edible insects commercially available in Australia

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Goosey, chewy and a little like chicken? While the nutritional and environmental benefits of entomophagy (the practice of eating insects) are well-established, the sensory properties of edible insects remain poorly defined and are often misconceived among Western consumers. To enhance Western consumer familiarity with insects as food and facilitate continued growth of the emerging edible insect industry, further research is needed to better describe the flavour and texture of commercially available edible insect species. A sensory lexicon comprises standardised vocabulary for the sensory attributes of a given food product category. Presented visually as a sensory wheel or as a list of terms, lexicons are useful in product development, quality assessment and marketing, as well as guiding sensory profiling for research. This study sought to establish a lexicon for edible insects that are commercially available in Australia. Crickets (*Acheta domesticus*), green tree ants (*Oecophylla smaragdina*), mealworms (*Tenebrio molitor*), superworms (*Zophobas morio*) and tyrant ants (*Iridomyrmex* spp.) were prepared using various cooking and preservation techniques, then evaluated by a trained descriptive analysis panel. Aroma, flavour and texture attributes were identified and defined by panellists to generate a standardised vocabulary. The vocabulary was then categorised and ordered to generate a sensory wheel. The edible insect lexicon and sensory wheel were refined in consultation with consumer and industry focus groups, and used by the panel to generate sensory profiles. Applications for these outputs in research and industry will be discussed, along with recommendations for further development of sensory communication tools and descriptors for edible insects.

'I am Flexigan': identifying pioneers groups for insect consumption

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Despite the benefits of integrating edible insects in human diets, their consumption is still not common in Western countries and successful implementation in markets is yet to occur. Many studies have examined factors affecting consumer behaviour towards insect-based food. However, an ideal target group has not been identified. This study investigated the diet motives of the different meat avoider groups (vegans, vegetarians, pescatarians and flexitarians) in Germany and their willingness to consume insect-based products in order to define a potential pioneer group for insect consumption. First, an online survey with 181 meat avoiders was conducted to identify drivers of consumption. After that, interviews were conducted based on consumer desires, motivations, neophobia on such groups. Through STP strategy, the survey respondents were divided into the sub-segments: ethical motivated, health and environmental meat avoiders. Ethically motivated meat avoiders were not a relevant target group for insect consumption, due to their general rejection of the consumption of animals, as they proved to be particularly strict in their lifestyle and fundamentally opposed to the consumption of living beings. Health and environmentally motivated meat avoiders were identified as not generally against the consumption of animals, open to the consumption of insects. We have been these last two groups defined as flexigans. The definition includes all different variations of vegetarian diets instead of solely focusing on a single approach. Flexigans proved to be neophilic and open to unfamiliar products. Therefore, they could be classified as either pioneers or early adopters. Marketing approaches are discussed based on the tendencies that became visible through the primary research.

Navigating the governance and commercialisation pitfalls in edible insect informal crossborder trade

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Mopane worm is an edible caterpillar (*Gonimbrasia belina*) traded and consumed widely in southern Africa. Its popularity has spurred trade providing important income source for harvesters and traders while meeting crossborder demand in the region. This growing popularity and commercialisation have driven an unprecedented demand for access to harvesting in Botswana and stiff competition across markets in South Africa. However, mopane worm regulation has been marred by inconsistencies, incapacity and regulatory constraints that result from imposition of inadequate and poorly designed regulatory tools, placing unprecedented pressures on mopane worm users. Other constraints across markets, including the exclusionary practices had multiplier effect on the threats to ecologically-sound harvesting practices and sustainability mopane worm populations, with significant implications for resource governability and actors' livelihoods. Using multi-sited ethnography and case study approaches, the study investigated and analysed the governance and commercialisation arrangements; and the strategies used to navigate these governance and commercialisation constraints. Significant regulatory constraints resulted from the combination of rigid regulatory tools and limited capacity of the institutions to ensure consistent application and compliance. Among harvesters, this undermined compliance with regulations due to poor enforcement and dissuaded compliant actors as transgressors were unpunished. Similarly, the challenges and costs resulting from gatekeeping, elite capture, and marginalisation of informal traders were experienced across markets in South Africa. Navigating the regulatory and informal trading constraints required crafting adaptive strategies. Owing to these constraints, and out of agency of the actors, informalisation, forum shopping and adoption of precarious practices were preferred across the trade chain. These adaptive strategies arose to mitigate effect of the everyday challenges resulting from regulatory inconsistencies, gatekeeping, elite capture, and marginalisation of informal traders as experienced across markets.

Obstacles to accepting insects as human food in three nations – Australia, Germany and Japan

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Once discovered using trial-and-error and adopted as food by our ancestors, insect consumption nowadays is still a strategy for the survival of many. Eating insects seem to have a variety of positive effects on the environment. Insects show an attractive nutritional profile, bearing health and other benefits. However, they are not part of the perspective of human food, particularly for the western world. People's resistance to insect-eating there is deeply rooted. Understanding barriers to eating more or fewer insects is paramount for finding solutions to future food security, and especially for implementing the idea of insects to feed the world. This survey-based study between three countries, namely Australia, Germany and Japan, demonstrates anthropological and psychological perspectives of people's food choices being constrained to their own environments and influenced by their eating behaviour (both acceptance of and reluctance to insects as food) by societies and cultures. The survey responses of the 20–60 years old participants were analysed to help recognise signs of willingness and readiness across the three nations toward insect consumption. The research focused on how often people made statements pertaining to disgust or the yuck factor and whether it is the biggest obstacle to insects acceptance as human food. However, the survey insights accentuated the prominence of the disgust factor as a mediating factor in the acceptability of insects as human foods, the responses brought into light other insightful country-specific areas of concerns such as the issue of clean-unclean, the role of gender and masculinity, and the insects masking under another form. Research examining these aspects in the three countries studied is limited.

Insect-Fed: an alliance for the promotion and certification of insect-based foods and feeds

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It is estimated that agriculture will need to increase 60% by 2050 in order to meet the demand of a growing world population. In particular, due to rising incomes, demand for animal-based proteins is increasing, adding pressure onto agricultural systems to provide ingredients for animal feeds. The only way to achieve the Sustainable Development Goals of the UN's 2030 Agenda, is by shifting to more sustainable products in the agri-food sector. Insect-based products are widely recognised as a potential solution for global food security, as they represent a more sustainable option for human food and livestock feed. It is possible to produce insect-based products with a much lower environmental footprint than conventional livestock and crop production, using significantly less land and feeding insects on organic waste, they prevent the emissions of methane. According to the Intergovernmental Panel on Climate Change, cutting methane emissions by 40% by 2030 would help limit global warming to 1.5 °C, avoiding the most extreme effects of climate change. The development of edible insect farming and production has been supported by FAO for the past two decades. A wide range of insect-based products have been introduced into the market, and this number is expected to increase in the coming years. Aware of the need to both communicate the life-cycle impacts of our food and promote the attributes of insect-based products, several stakeholders of the insect farming community – including producers and academia – have been brought together in the Insect-Fed Alliance. The main objective of this alliance is to create a collaborative platform that provides information and spreads knowledge about insect-based products to help consumers make informed choices. To achieve this, a certification scheme is being designed, with the aim to recognise insect-based products that fulfil a set of high-level sustainability and quality criteria. These products will carry the Insect-Fed Label, to account for their sustainability performance and, through the use of technology, consumers will be able to track the environmental impacts throughout its life cycle.

North American consumer acceptance of insect feed ingredients in pet food

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Insects for the use in feed and food is becoming a widely talked about solution to help feed an ever-increasing protein hungry world. While the science is providing promising results in the form of insect husbandry, production technology and the ability to scale up production to increase output, is North American culture ready to accept the concept of insect ingredients and especially in pet food? It is time for the commercial aspect of the industry to catch up to the science or technical component. As part of a quest to understand the views of the North American pet food consumer, a multi-year study is concluding in the spring of 2022. The outcomes of the study will reveal the thoughts, opinions and actions of dog and cat owners towards feeding their pets an insect-based diet. It will be the consumers of insect-based products that will determine the success of our industry. What are the key opportunities and hurdles for our industry to address so that we can take the next step to having insect ingredients provide new and novel solutions? Results from an on-going pet food consumer tracking study on insect ingredients will provide insights so that collectively we can understand the voice of the end use customer and determine the opportunities, challenges and a collective path forward.

Updated insights on marketing of insect as feed focused on insect-based pet food applications

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Insect farming as feed has arguably been continuing its coming of age journey in the last 2-3 years with more and bigger commercial production facilities coming on line across the world. While global production volumes have increased, they still constitute only a very small fraction of the global feed protein demand. At the same time, insect producers have developed different applications and markets for their farmed insect as feed products. An application that has seen a notable growth is the nascent insect-based pet food category. Originating from products in Europe & North America, insect-based pet food has evolved at the intersection of insects as food and insects as feed with a focus on dog food, and to some extent cat food, and today presents a significant market opportunity for farmed insect products globally. In our study, we evaluated the global insect-based pet food market, surveyed brands and product formats, evaluated value propositions and messaging and compared them against published literature findings, analysed the actual use of insect ingredients as part of the final product formulation and assessed pricing and distribution practices. Being based in Asia, our study included a geographic segmentation and evaluation of consumer perception and preferences in South East Asia.

Insect school: a way to move the BSF industry forward

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The insect industry is known for its high potential, but it's also known as a closed industry for the pioneers who want to start. When everybody keeps thinking they are sitting on the 'golden idea' then nobody will move. That also means the industry will not move forward. To make the industry move you need to offer something which triggers the people to move. The Insect School is set up with that goal. To create a meeting place where people can go to share knowledge and to obtain new knowledge. It's proven that a physical location has a very positive effect on co-operations in an industry. At same time setting it up has been facing the same difficulties because nobody wants to make that first step. So this step is taken by 1 person in 2021 and from 4 April 2022 the practical location in America (don't be fooled, this is not the USA but a village called America located in The Netherlands!) is opened and accessible for all who have an interest in the BSF industry. From people who want to start, growers who want to experiment, feed trials, university research, technical developments, breeding, frass trials, etc. Many universities are involved with the BSF industry and perhaps will say now but we already do our own research at our own university. Exactly! That we all should cherish and promote. The idea of the Insect School is actually the translation between the universities and commercialisation. It's that in-between step in the proof of concept on pilot scale before going large. From 1 till 20 tons of organic waste production rooms. That is good for commercial testing and showcasing investors your proof of concept. The physical location in America (Netherlands) is the practical centre. The online version www.insectschool.com is also being build and when IFW2022 takes place it will be online. It should be the information source for BSF farming. At same time training courses by universities and commercial companies can be shown so people are guided in their journey into BSF farming. This initiative might become for more insects in the future but first things first.

Farmers' willingness to pay for commercial insect-based poultry feed attributes in Kenya – a choice-experiment method

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High feed costs make chicken production prohibitive especially in developing countries owing to overreliance on protein feed ingredients such as soybean and fishmeal that are characterised by rising food-feed competition and supply chain impediments such as restrictive border policies and the recent Covid-19 pandemic. The use of insects such as the black soldier fly (BSF) as a sustainable alternative protein source has attracted global attention recently. However, there is a dearth of empirical insights on farmers' preferences for commercial BSF-based feeds. This study evaluated farmers' willingness to pay for attributes of BSF-based commercial poultry feed in Kiambu County, Kenya using a choice experiment and the random parameter logit (RPL) based on a survey of 314 predominantly poultry farmers. Results of the RPL show that 93% of the farmers are willing to pay for BSF-based feed and premium prices range between US\$0.34 and US\$3.39 for BSF-based feed in the form of either pellets or mash, feed explicitly labelled as containing insects, feed mixed with soybean or fishmeal and dark-coloured feed. This is a pioneering study that specifically values commercial feed based on various components and combinations. The findings provide evidence for multi-stakeholder collaborations to facilitate the creation of an inclusive BSF-based feed regulatory framework for sustainable feed and poultry production.

Farmers' perceptions of commercial insect-based feed for livestock production in Ghana

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Insects have been proven to be potential alternatives to animal and plant protein sources worldwide. Although insects occupy 80% of the global biodiversity and have been part of traditional delicacies for over two billion people, they are among the most underutilised feed resources. The sustainable utilisation of insects in livestock feed formulation has the potential to transform the current overreliance on fishmeal and soybean meal to a vibrant circular economy that offers employment opportunities especially for youths and women at the grassroots with effective feedbacks to the environment. This study evaluates poultry farmer's perceptions of commercial insect-based feed (IBF) products and assesses the factors that would influence its adoption. Principal component analysis used to develop perception indices that are subsequently used in multiple regression analysis of survey data collected from a sample of 400 farmers. Over 95% of the farmers were ready and willing to use insect-based feeds. The Principal component analysis identified feed performance, social acceptability of the use of insects in feed formulation, feed versatility and marketability of livestock products reared on insect-based feeds as the key attributes that would affect farmers' purchase decisions. Awareness of insect-based feeds attributes, group membership, off-farm income, wealth status and education significantly influenced farmers' perceptions of IBF. Interventions such as experimental demonstrations that increase farmers' technical knowledge on the productivity of livestock fed on insect-based feeds are crucial to reducing farmers' uncertainties towards acceptability of insect-based feeds. Public partnerships with resource-endowed farmers and farmer groups are recommended to improve knowledge sharing on insect-based feeds.

Quality of edible insects seen by Belgian academics

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In Belgium, as everywhere in Europe, edible insects are not yet introduced in food habits. Edible insects must reach a certain level of quality to be accepted as food by European people. Quality is defined as ‘the requirements necessary to satisfy the needs and expectations of the consumer’. In the case of food, quality is divided into five dimensions: safety (i.e. hygienic quality), health (i.e. nutritional quality), satisfaction (i.e. organoleptic quality), service (i.e. functional quality) and society (i.e. symbolic quality). Each dimension has a variable importance depending on the consumer and the food. A study was conducted in Gembloux (Belgium) at Gembloux Agro-Bio Tech – University of Liège (GxABT). 475 respondents (mean age: 27.62±11.45 years old) took part in the online survey voluntarily. This survey aimed to collect general information about entomophagy (e.g. consumer acceptance, experience of eating insects, etc.) and to define the most important quality dimension relative to entomophagy. This study was also an opportunity to challenge the questionnaire which is the initial version of a questionnaire for further studies. More than 60% of academics from GxABT stated they would be willing to eat edible insects and they have already experimented insect eating. Most respondents knew where they could buy this food, mainly on the Internet or in store. Concerning the quality of food, health was considered as the most important dimension followed by society and satisfaction. In other words, respondents wanted to eat a healthy food with edible insects.

Insect-based feed for aquaculture products: an investigation of Italian consumers level of consciousness and acceptance

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Consumers' interest in sustainability and the environmental impact of food production is becoming increasingly important for their consumption choices. The combination of these two factors prompted the sector of aquaculture to find more sustainable alternative sources for animal feeding as a possible action to mitigate environmental impact of this sector. Insects have already proved to be a valuable alternative source to common feed in aquaculture. However, fish farmed with insects is still perceived as an unconventional product. In addition, the investigation on consumer behaviour on animal origin products fed with insect-based feed is still in its infancy. But is the Italian consumer truly ready for this innovation? This study aimed to understand Italian consumers' attitudes towards the new trends of fish fed with insect-based feed. An online survey was conducted between January and March 2021; it was web-programmed using Google Forms and developed in the Italian language. The survey was completed by 209 Italian consumers aged 18-75 (49% male). Data on consumers' diet, purchasing habits and awareness degree of topics regarding fish diet, feed quality, sustainability, and food neophobia were collected. The final dataset was analysed using descriptive statistics methods. The effect of diet, neophobia, attitude towards sustainability, purchasing behaviour, knowledge about aquaculture sector, age, and gender on the importance consumers give to the quality of feed, the climate change, buy more sustainably and to the sustainability of insect-based feed was estimated by ANOVA one-way models. The higher is level of consciousness concerning the variables analysed, the higher is the willingness to buy and eat innovative products. A higher knowledge degree can also be found in younger and female consumers who pay more attention to the quality of feed and the environmental impact of its diets. The test sample seems to be ready for this innovation but needs proper information.

Market opportunities of BSF insect frass

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Black soldier fly larvae insect frass (BSFL) is gaining popularity as high value fertiliser in professional and consumer end markets. End markets are interested in insect frass due the sustainability aspects (low footprint) and functional benefits. Most important functional benefits of frass are the stimulation of beneficial soil micro-organisms, a high percentage of organic matter and the supply of essential nutrients. Currently there are several market applications for insect frass. Frass is applied in professional markets like vegetable cultivation, flower cultivation and golf/sport fields. Another market is the consumer market, Frassor (brand name) is an example of a insect frass based consumer product. The consumer market is relatively more emotional driven then the professional market. To further unlock these frass markets, several things are needed. Most important is research to the functional benefits of insect frass to get a better understanding of the real value of insect frass. Secondly it is important that legislation for this new animal by-product becomes clearer. Finally, it would be useful if governments started to stimulate the use of frass through, for example, subsidies. Methods used to generate results: We used a combination of literature studies (studies since 2018 on topics related to insect frass and organic fertilisers) and questionnaires from Protix insect frass users.

Level of the concreteness of insect information on packaging and impact on product preference and evaluation

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Our research questions the impact of the depiction of the insect on the packaging as well as its association with other ingredients on the behaviour and evaluation of products in the context of the purchasing environment. Using a virtual reality experiment, reconstituting a shop shelf of four apple-caramel cereal bars and insect flour, we test the impact of different graphic depictions of insects providing an abstract or concrete level of information about the insect (drawing vs photo) when it is mixed with other ingredients, notably fruit (cut vs whole). Through this study conducted with 44 participants in a first stage of manipulation of the products within subject using virtual reality, we show that holding the product allows a better attention of the product by integrating the level of proxemia achieved with the product (the fact that the product is close to the face or not) and the time spent looking at the front of the packaging (FOP). Self-reported participants preferred the products to whole fruit designs, with no significant difference between insect designs. However, the integration of behavioural measures from the virtual reality experience, are additional explanatory predictors to the increase in perceived realism on product preference based on the insect design on the package. In this way, the bar with the photo compared to the drawing, resulted in increased attention (bringing the bar closer to the face) inducing an increase in choice for the bar with the photo. We also note that the FOP was observed for longer with the drawing than with the photo and that this increased the choice of bar with a whole apple and a drawn insect. Then, in a second step we studied the perceptions of these two insect designs chosen in terms of perceived novelty and originality, perceived healthiness and taste evaluation of the product when tasted. Our results highlight marketing levers for designers to make new insect-based products more easily accepted by the packaging.

Biotechnology & non-food applications of insect

Functional properties, bioactivity and ionic dye absorption of chitosan produced from house crickets with novel methods

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House crickets are considered as a good source of chitin. Chitosan, the product of deacetylation of chitin, has various applications in the food and non-food sector. Chitin can be extracted from crickets via a sequential deproteinisation (NaOH, 1 M, 80 °C, 22 h) and demineralisation of the material (HCl, 1 M, 98 °C, 2 h). This process is time-consuming and requires hazardous chemicals that can be pollutants and affect the properties of chitin. Two alternative processes were introduced for chitin extraction from house crickets. The first process combined fermentation with *Lactococcus lactis* (30 °C, 7 d, *Lactobacillus* broth) and enzymatic treatment with bromelain (5 h, 60 °C, pH=5.5). The second approach implemented microwave treatment to accelerate the chemical process in a total time of 16 min. All isolated chitinous materials were deacetylated. The chitosan samples generated from the chemical, biological and microwave-assisted process showed comparable chitosan content (91.85, 90.31 and 87.64%), but different water- (991.50, 772.51 and 1,379.62 g water/g chitosan) and fat-binding capacity (1,078.62, 885.30 and 860.92 g fat/g chitosan). All samples showed antioxidant activity comparable to commercial chitosan, with required concentration of 5 mg/ml to achieve a 50% of antioxidant activity. Finally, the absorption of methylene blue from water, as a physical process for wastewater detoxification, was tested for all samples, which showed significant differences. The chitosan obtained through the biological process showed an 85% absorption from a 10 mg/kg solution after 5 min, while successfully absorbing 70% of a 320 mg/kg dye solution. The results suggested that both alternative process are sufficient to replace the chemical process for chitin extraction.

Evaluation of chitin extraction with a green method from exuviae and imago matrix from black soldier fly breeding

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In 2015, the worldwide chitin production was set up to 28,000 tons while the request was around 60,000 tons. Moreover, the chitosan market value was estimated around 1,205 million \$ in 2015, with a growth rate of 15.4%. It will reach 6,38\$ in 2024. In this scenario, the exploitation of new chitin sources can be interesting for the market. Exoskeleton of insect is rich in chitin. The insect sector grew up in the last 10 years. The importance of the sector lies in the possibility of recovering a lot of waste and by-products and bioconverting them in new products. The main markets related to the insect sector are the use of insect as feed, as food and in the production of biomaterials. Considering the insect sector, the by-products deriving by the reproduction of some species can be exploited for chitin production. Black soldier fly (BSF) is one of the most promising species and the *exuviae* (BSFE) and *imago* (BSFI) from breeding room are one of the most profitable for chitin extraction. The aim of this research was to evaluate the feasibility to extract chitin from BSFE and BSFI with a new green technique with lower environmental impact. An innovative patented process was used for the chitin extraction from BSFE and BSFI. The key point is the application of DES (acronym for deep eutectic solvent), a new class of green solvents reported in the literature. The reaction conditions are mild (room temperature and short time). By a simple filtration the chitin is recovered. The chitin is then characterised by x-ray powder diffraction and thermogravimetric analysis (TGA). From 1 kg of dried exuviae (moisture of 9 wt %), 300 g of technical grade chitin are obtained. The BSFE and BSFI are good by-products that can be exploited for chitin extraction and chitosan production. The market is still in early-stage but with the increasing of BSF production the availability of chitin will reach soon tons of by-products that can be exploited for the production of chitin with low environmental impact.

Biocontrol potential of black soldier fly (*Hermetia illucens* L.) frass against *Fusarium* and *Verticillium* wilt of tomato

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Fusarium oxysporum f.sp. *lycopersici* (Fol) and *Verticillium dahliae* are globally destructive fungal pathogens of the tomato plant (*Solanum lycopersicum* L.), causing significant economic losses to farmers. Current chemical treatments against these fungal pathogens are often unsafe, expensive, ineffective, and detrimental to long-term soil health. Using organic biofertilisers, such as black soldier fly (*Hermetia illucens* L.) frass (BSFF) has been shown to have some beneficial suppressive effects on the development of a range of soil-borne phytopathogens *in vitro* and *in vivo*. Soil-borne disease suppressive activity would provide an added value to insect frass-based biofertilisers, as it is a desirable property both for organic amendments and for organic growing media. In a tomato growth trial using frass amended commercial compost, this study assessed the impact of BSFF application on soil microbiome diversity in bacteria and *fungi* using DNA metabarcoding. Tomato trials were also undertaken to quantify the growth of soil-borne pathogens, *F. oxysporum* f.sp. *lycopersici* (Fol) and *V. dahliae* using real-time polymerase chain reaction. The study hypothesised that BSFF can facilitate the growth of plant promoting rhizobacteria and inhibit fungal phytopathogen growth in the soil. The results are suggestive that BSFF application to soil can stimulate plant growth-promoting rhizobacteria while potentially reducing fungal disease incidence and disease severity. BSFF provides an environmentally sound addition to the tools farmers utilise in integrated pest management and is ideal for use on organic farms and in glasshouse settings where pesticides are strictly controlled. Further trials should assess the impact of BSFF on a range of phytopathogens in an open field setting.

Influence of residues from black soldier fly (*Hermetia illucens*) rearing on the plant-associated soil microbiome

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The larvae of the black soldier fly (*Hermetia illucens*) efficiently close resource cycles. Next to the nutrient-rich insect biomass, the frass produced in the process is a promising plant fertiliser. Besides a high nutrient content, frass contains a diverse microbial community and application to soil can potentially promote soil fertility and agricultural production through the introduction of beneficial microbes. This research assessed for the first time the application of frass on plant-associated bacterial and fungal communities in the rhizosphere of a grass-clover mix in a 42-day greenhouse pot study. Untreated frass from a Rwandan bioconversion company, the same frass sterilised by high-energy electron beam (10 MeV; >32 kGy), an organic compost were applied to a silt loam soil at a rate of 150 kg N/ha and compared to an unamended control soil. Soil bacterial and fungal community in both source and soil at different times after fertiliser application was assessed by high-throughput sequencing of ribosomal markers. Additionally, soil respiration indicative for microbial activity, plant yield and soil physicochemical properties were analysed. Results show that frass application especially shaped the soil fungal community. Particularly taxa associated with decomposition of organic matter (e.g. *Mortierella* spp.) were promoted by frass application. Our findings suggest that biotic as well as abiotic components of frass impacted soil fertility and plant performance (+17% yield vs unamended control). Our research emphasises that closing the nutrient loop of the food system by frass application offers versatile opportunities for sustainable agriculture.

Nutrient quality and maturity status of frass fertiliser from nine edible insects

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Globally, there is growing interest to recycle organic waste using insect larvae into high-quality frass fertiliser through circular economy approach. However, focus has largely been on black soldier fly (BSF) and mealworms, thus leaving a knowledge gap in the exploration of frass fertiliser products from other farmed insects. This paper presents the first comparative report on the nutrient concentrations, fertilising indices (a measure of fertiliser quality based macronutrients, respiratory activity and C/N ratio), nutrient supply potentials and compost maturity of nine edible insect frass fertilisers. The frass fertilisers generated by different insect species were air dried and ground into fine powders which were used for analysis of macro, secondary, and micro nutrients as well compost maturity indices (pH, electrical conductivity, germination index, C/N ratio, ammonium and ammonium/nitrate ratio) using standard procedures. Our results revealed that frass from all the insect species had adequate concentrations of nitrogen (N), phosphorus (P) and potassium (K) for optimal crop growth. The fertilising indices of the frass fertilisers were above 3, indicating high suitability for use as organic fertiliser. However, BSF frass fertiliser had significantly higher N (by 20-130%) and K (by 17-193%) concentrations compared to others. The P concentration of *Gryllus bimaculatus* frass fertiliser was 3-800% higher compared to those of frass fertilisers from other insect species. The potential N and K supply capacities of BSF frass fertiliser were 19-78 and 16-190% higher than those of other insect frass fertilisers, respectively. The P supply capacity of cricket frass fertiliser was 17-802% higher compared to others. The highest germination index (267%) was observed in seeds treated with BSF frass fertiliser. Frass fertiliser obtained from the other eight insect species showed medium to high phytotoxicity. These findings demonstrate that insect frass fertilisers are promising alternatives to existing commercial fertilisers (i.e. mineral, and organic) for improved soil health and crop production.

Mealworm frass, chicken manure and municipal compost effect on the growth and yield of vegetables, herbs and flowers

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Insects represent a great protein alternative to traditional animal protein and their farming yields fertilising manure. An experiment was conducted to assess the agronomic potential of *Tenebrio molitor* L., 1758 frass on plants. Tests were done on five vegetable species (beet, carrot, kale, radish and sweet corn), three flowers (dwarf sunflower, *nasturtium* and *zinia*), two fruits (cucumber and tomato), one grass (arugula) and one herb (basil). Each plant was grown, in triplicate, with three different treatments in geotextiles pots on the roof of Centrale Agricole Coop (Montréal, QC). All treatments included 5% compost enriched substrate (control), plus 0.5% (v/v) frass (treatment 1) or an isonitrogen concentration of hen manure (treatment 2). Growing parameters such as germination, total height, stem diameter, number of flowers, number of fruits and harvested biomass were monitored for 91 days. Nitrogen fertilisation increased edible biomass harvest by an average 16-fold in comparison to the control. Frass and hen manure yielded equivalent results with 1.14 times more leaf biomass and 1.2 times more for root biomass with the frass fertilisation. Both manures inhibited early germination in beets and carrots, but did not negatively impact edible biomass production. Furthermore, plants grown with the use of frass have shown larger flowers than the control. These results not only bring forward the potential of insect frass as fertiliser, but also suggest an opportunity for insect producers to commercialise frass, an inevitable by-product of insect rearing, and generate revenues.

Decomposition ecology as a guiding framework in the design of black soldier fly production systems

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The production of black soldier fly (BSF, *Hermetia illucens* L. 1758) has emerged over the past decade as a novel way to create animal protein. Black soldier fly production differs from existing animal rearing systems in that BSF are saprotrophic organisms that are reared inside of a high bioburden feed. Moreover, in this system the feed is not an inert input, but rather a matrix that sustains a diverse biome of which BSF are but one possible successional step. Insect-driven changes in feed composition and microbiome take place during rearing, while other successional players such as saprotrophic microbes can facilitate BSF substrate utilisation, and players further downstream can process BSF waste. Unlike existing livestock rearing systems, wherein the individual organism is considered the production unit, in a BSF system the production unit consists of both insect larvae and the associated microbiomes (collectively referred to as the necrobiome). With this mindset, for a given rearing container the producer manages both the larvae and the associated microbiomes via manipulation of variables such as initial feed composition, initial microbe inoculation, feed handling, and rearing temperatures. This framework allows the producer to leverage on synergistic ecological interactions that occur between the insect larvae, the feed matrix, and the microbial players. We build on that basis to demonstrate further cases of how decomposition ecology can be used as a framework to design more efficient BSF rearing systems which can be manipulated to optimise different aspects of the production system, from larvae nutrition, residual frass composition and production of organic volatiles.

***In vitro* antifungal activity of Gainesville diet against plant pathogens? – a cautionary tale!**

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Black soldier fly (*Hermetia illucens* L.) larvae (BSFL) can bioconvert a wide variety of organic residues into larval biomass with high nutritional value. A by-product of this bioprocess is frass which is composed of larval excrement, larval exuviae, and undigested feedstock. Previous work in our laboratory has demonstrated antifungal activity of aqueous frass extracts against a range of phytopathogens. For this work, a reference laboratory diet 'Gainesville house fly diet' (50% wheat bran, 30% alfalfa meal, and 20% cornmeal) was employed. Triplicate samples of four-day old BSFL (n=800) were placed in plastic containers with 800 g of Gainesville diet (70% RH) and kept at 27 °C under dark conditions for 7 days. Samples of frass were removed and assessed for *in vitro* antifungal activity using the double-layer agar assay. Control containers received Gainesville without BSFL and were incubated and sampled identically. Intriguingly, *in vitro* antifungal activity against the phytopathogens *Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora capsici*, *Fusarium oxysporum*, *Alternaria solani*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea* was found in frass as well as the control. Subsequent investigations demonstrated that freshly hydrated Gainesville also showed *in vitro* antifungal activity. When dietary ingredients were tested individually, the alfalfa fraction revealed a strong antifungal activity. Microorganisms were then isolated from the Gainesville diet by standard serial dilution plate technique and the antagonistic activity of each isolate against the above-mentioned phytopathogens was investigated. A bacterium with strong antifungal activity was genetically identified by 16S rDNA with 98% similarity to *Bacillus* spp. This work underlines the potential importance of feedstock inherent microbial characteristics on antifungal activity and should be especially considered when demonstrating the properties of frass for potential use by industry.

The effects of frass on soil pH and phytoremediation capacity of willows in potting substrate and contaminated soil

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Yellow mealworm (Tenebrionidae: *Tenebrio molitor* L., 1758) farming generates 2–4 kg of frass for every 1 kg of edible insects. In a circular economy context, this frass with an N-P-K ratio of 3-3-2 can be used as organic fertiliser. Frass also helps plants tolerate stressful abiotic conditions. Brownfields with low drainage, low nutrients, and alkaline pH can limit plant growth. Salicaceae plants, such as willows and poplars, can tolerate these conditions and phytoextract contaminant trace metals. By improving biomass production, fertilisers boost phytoremediation potential. Unsustainable mineral fertilisers may be traded for renewable organic ones, but many like compost and biomethanisation digestate contribute to increase soil pH, which decreases trace metal bioavailability for phytoextraction. We hypothesised that frass supplements could lower soil pH to and increase Cu extraction. In a preliminary greenhouse experiment, we planted willow (*Salix miyabeana* SX67) cuttings with 0–4% v/v frass (equivalent to 0, 61.7, 123.4, 246.7, 493.4 and 986.8 kg N/ha) in clean substrate (Pro-Mix) and contaminated soil (5 l pots). Intermediate fertilisation values (0.5 to 1% v/v) yielded the significantly longer stems and higher dry aerial biomass (Pro-Mix). After 1 and 2 months, an increase in frass amendment (0 to 4% v/v) significantly decreased pH (8.0 to 7.4 in soil and 7.6 to 7.0 in Pro-Mix). Based on these results, a contaminated soil experiment in a growth tunnel compared isonitrogen doses of frass to chicken manure, biodigestate and an unfertilised control (250 kg N/ha). Frass yields significantly taller plants than the control, with less variability in results than for digestate and hen manure. Enhanced biomass and decreased pH suggest we may find heightened Cu uptake in plants. To our knowledge, this is the first experiment testing frass in phytoremediation contexts of contaminated soil.

Circular economy & environmental sustainability

An automated, modular system for organic waste utilisation using *Hermetia illucens*: design and sustainability

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Food waste is one of the crucial problems of modern food systems. Methods to utilise food waste into valuable biomass of defined composition automatically and in decentralised locations are lacking. This study presents a modular design for agri-food residue utilisation and continuous production of insect *Hermetia illucens*. A life cycle and economic assessment are carried out on the hypothetical design to define whether the proposed system can be ecologically and economically viable. Modular design is modelled using experimental literature data on *H. illucens* growth on food waste as well as performance data of machinery utilised for grow and processing. The data became the basis for the Life Cycle Inventory for LCA and economic assessment. Producing one kg of dried biomass would cost 5.3 € with a potential to reach 3.55 €, being a break-even price paying back the investment costs after five years. It is also associated with 2.77 kg CO₂ eq. emitted (with potential for reduction to -1.32 kg CO₂ eq.), 55.2 MJ of non-renewable energy use (reduction to -13.14 MJ), and 0.68 m² of arable land occupation (reduction to -0.023 m²). Avoidance of waste treatment is considered as a reduction potential in the study. The results indicate that insect biomass produced with proposed modular system can be beneficial if compared on a protein basis to benchmark protein sources (meat and protein sources). Furthermore, environmental and economic performance of decentralised production of insect can be improved via the avoidance of waste treatment and drying (most impacting process). Application of fresh insect biomass for further purposes then should be considered.

Unleashing the power of insects, a life cycle analysis review of Entosystem

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Insect proteins are a viable alternative to meet the growing worldwide demand for food and feed. The use of the black soldier fly (BSF; *Hermetia illucens*) as a tool to transform and upcycle pre-consumer food waste has been growing in popularity due to its high efficiency. Here we present an ISO14064 life cycle analysis of Entosystem's future production facility based on three years of representative data from a pilot plant in Sherbrooke (Québec, Canada). The LCA quantified the environmental benefits in terms of greenhouse gas emissions, atmospheric contaminants, and soil and water benefits. When compared to the production of fish meal, the production of synthetic fertilisers and the treatment of food waste through composting and landfill, Entosystem's technology shows a reduction of close to 41 ktCO₂e of GHG emissions per year in Canada. This represents a reduction of 89% in GHG emissions when compared with the baseline. Other environmental benefits of this project also include landfill avoidance, organic waste recovery from commercial and industrial sources, water savings and reduction in phosphorus losses.

Aquaculture waste valorisation with black soldier fly larvae: a circular approach

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Aquaculture wastewater is a nutrient rich material, which may present a severe problem for the environment when not disposed properly. Black soldier fly (BSF, *Hermetia illucens*) has been described for playing a crucial role in the circular economy by upgrading several kinds of biological waste materials into valuable nutrients. In this study, aquaculture sludge obtained after concentration of aquaculture waste and mainly consisting of uneaten feed and fish faecal drops was tested for BSF larval rearing. Seven substrates were obtained by mixing different proportions of sludge with commercial chicken feed (control). Larval growing performances as well as chemical composition of substrate, insects and frass were recorded. Larval survival and growing index did not show significant differences between the tested substrates. Final larvae average weight and conversion efficiency increased by increasing the fish sludge in the substrate, with highest levels reached at 75% of inclusion (132.71±12.48 mg and 0.76±0.05%, respectively). Dry matter content of larvae grown on substrates containing up to 50% of aquaculture sludge did not show significant differences, while higher dry matter (39.53±1.00% of fresh weight) was recorded for insects raised on 75% of sludge. No significant differences were observed on ash and total fat content for larvae reared on the different mixtures of sludge and chicken feed. Nitrogen and non-fibrous carbohydrates contents were higher for larvae growth on 25% of sludge (10.19±1.16 and 4.20±0.57%) compared to insects raised on 75% of sludge (7.12±0.17 and 4.20±0.23%). Insect development promoted mineralisation of the sludge organic matter, with strong correlation observed between Carbon to Nitrogen ratio of the insect frass and percentage of sludge in diet ($r=0.97$). In conclusion, BSF show great potentiality in upgrading aquaculture waste. However, further studies are needed focusing on improving growth and conversion performances as well as larvae nutritional composition, by identifying the best inclusion level of aquaculture sludge in the insect diet.

Black soldier fly as a tool for an advantageous system of a local circular economy

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Every year, around one-third of the food produced for human consumption in the world is lost. It consists of biomass rich in nutrients that the traditional ways of disposal not fully valorise. Black soldier fly (BSF) can convert organic wastes into nutrient-rich raw materials with a new approach of sustainable circular economy (CE). This study evaluated BSF as tools of biomass valorisation, creating a system of local CE. A census of the most representative agro-food companies in the area was selected and contacted. A database was created concerning by-product (BP) categories, quantities, seasonality, their current management. BP were evaluated in terms of suitability for BSF, analysing their bibliographic evidence towards the breeding of BSF considering the nutritional quality and the quantity surveyed. The 115 companies were divided into 6 categories: brewery (BRE), milling (MIL), fruit-vegetable (FRU-VEG), rice (RIC), winery (WIN), distillation (DIS). A total biomass of 87,325 tons was recorded. The major contributors were represented by MIL (81,225 tons), DIS (3,710 tons) and FRU-VEG (1,190 tons) BP. Only a few of the BP identified were recognised as suitable for BSF rearing: BRE, MIL, FRU-VEG, WIN, DIS. Only 0.37% of the BP was inserted in the process of CE, 0.30% was managed through the combination of agricultural and CE principles, while 97% was used for agricultural-zootechnical purposes. Furthermore, 1.37% of the BP (1,201.8 tons) was disposed as waste. There was a relevant quantity of BP present in the area under-valued. The management through BSF rearing would be more efficient and convenient than traditional way, as emerged from economical evaluation and comparison of the two scenarios. This bio-transformation technology can be scalable and customised, in relation to the geographic area under study and it contributes to the creation of a new source of protein addressed to the feed sector, reducing the under valorisation of BP and their management costs.

West African context call for rapid implementation of insect meal for fishmeal substitution

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In West Africa, numerous economies are dependent on fisheries resources; this sector can reach almost 10% of the national gross domestic product and represents a central sector for food security as the most important animal protein for human consumption and aquafeeds. Due to overexploitation, West African fish stocks are under serious threats and massive implantation of fishmeal factories has raised serious concerns about the sustainability of small pelagic fish resources. Although this situation is well understood by many political decision-makers, this trend is not about to be reversed. Concurrently, the government of Senegal seeks to increase the production of the Senegalese fish farming industry. To address these challenges, we are investigating the possibility of harnessing the ability of black soldier fly (BSF) larvae to replace fishmeal. Given the lack of formal institutional initiatives in Northwest Africa, we initiated in 2020 a collaborative program to establish a technical platform ‘Art Sunu Gueej’ to develop an inter-institutional insect production unit in a controlled environment. We have identified endemic BSF populations in local piggeries as well as agricultural surpluses including millet bran, rice bran, and mango waste that could serve as BSF feedstocks, to ultimately partially replace fishmeal for aquafeed formulations. We have established an Action Plan for awareness of political decision-makers, including a research plan to: (1) optimise and adapt BSF strains for West Africa production and, (2) test insect meals for aquaculture and poultry sectors. Based on these results, we will use our new platform for fundraising and foster collaborations to promote insect meals as fishmeal replacements for aquafeeds in West Africa.

Insect for feed vs waste management: company internal R&D perspective

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Looking at the insect industry from a circular economy perspective, two branches are being developed worldwide. The insect protein segment has been studied for a decade and the early attempts into taking the products into the commodity market are being pursued by companies across the globe. Insects as a waste management tool has been explored by academics but very few companies emerged with a market strategy that could harness the power of insects to flip the face of the unsustainable classic waste management approaches. Internal structure of R&D in companies in both segments are drastically different. Biological R&D in insect protein meal is mainly targeting production, genetics and farming process optimisation, making the gap between R&D and growth in a grey area. For waste management, R&D is a core component of growth and market exploration. In this presentation, we will map the differences between R&D strategies, pathways and goals with focus on the challenges of waste management using insects.

The basis and structure of new PRIMA project: ADVAGROMED

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The expansion and intensification of agriculture and animal production systems are major drivers of biodiversity loss through conversion of natural habitats to agricultural land, extensive use of chemical inputs (fertilisers and pesticides), and landscape homogenisation. Integrating agroecological practices with current agricultural farming systems could offer a sustainable means to conserve and enhance the endangered farming biodiversity and increase ecosystems services. The ADVanced AGROecological approaches based on the integration of insect farming with local field practices in MEDiterranean countries project aims to develop innovative and holistic food system based on agro-ecological principles and circular economy practices, and to increase the resilience of the agro livelihood systems. ADVAGROMED uses by-products from local insect producers to deliver different products. First, insect frass is used as bio-product to improve soil fertility, deliver plant protection effects and enhance soil microbial biodiversity; in this way the dependence on mineral fertilisers and chemical pesticides can be reduced. Second, live larvae are fed to local poultry breeds ensuring optimal animal performances, health and product quality; in this way the dependence of imported feeds can be reduced. Biodiversity is promoted at various levels, i.e. at farm level, by promoting the genetic variability of local crops and varieties/animal breeds, but also at a regional level by minimising the negative impact of chemical inputs on the microfauna through the exploitation of insect frass as bio-products for sustainable soil fertilisation and plant protection. This research receives the support of the ADVAGROMED project (Prima 2021 – Section 2).

Quality control, legislation & policy

Past, present and upcoming milestones on edible insects' regulatory journey

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In the contemporary era, entomophagy has long remained anecdotal in the Western world and despite a steady growth, the edible insect segment remains a niche. The potential contribution of the insect sector to the protein shift, and the promising potential of insects as a source of nutrients and functional food ingredients is however gradually opening more and more development opportunities for a wide range of products. Indeed, extensive research is conducted around edible insects, in all areas of nutrition and food technology. In the European Union, the policy agenda favours the development of innovative, more sustainable products including those that the insects sector can supply. The legal and regulatory environment plays an important role in the effective access of these products to market. Defining the pathway to market is an essential step in any product development, and insect-based food products are no exception to that rule. In this presentation, we discuss the regulatory status of a number of insect-based and insect-derived foods and ingredients in the EU, and we examine the requirements that must be fulfilled in order to place these products on the market. We then compare the EU framework with that of other regions of the world, including North America and Asia, in order to bring these into perspective and identify the opportunities and pitfalls built into the global regulatory framework.

Quality criteria of frass for agriculture uses

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The upcycling of organic materials via entotechnologies represents a real opportunity for ecological recovery for all kinds of residual organic materials. In Québec, but also elsewhere, the number of insect breeding farms is growing, and the resulting quantities of frass are also expanding. Although frass is an organic material with attractive fertilising properties for agricultural uses, an adequate regulatory framework is necessary to ensure the use and marketing of this product. The establishment of frass quality criteria would facilitate the evolution of frass regulations, particularly for production, processing, marketing and use in Québec. An assessment of quality criteria is being developed to meet standardisation issues. The quality criteria are based on the evaluation criteria set for fertilising residual materials and composts. The physico-bio-chemical properties of a range of frass produced in different ways and from different sources are analysed to classify frass according to its potential for agricultural uses. These criteria will make it possible to standardise the treatments of the frass according to the diet, the nature of the substrate and the type of insects. In addition, these standardisation criteria will be useful to insect farms, private industry, users, and government organisations.

The first novel food authorisation for insect: paving the way for the booming market of insect as food

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Insects as food are deemed very promising as a solution to address future food challenges. Paradoxically, while substantial innovation was emerging in this sector in the past years, its commercial development was limited, especially by a lack of official authorisation. European food regulation is one of the most stringent worldwide with a conservative approach. The Regulation (EU) 2283/2015 of the European Parliament and of the Council on novel foods which came into force the 1st January 2018 clarified the marketing of edible insects within the EU as a first step forward. However, it was not until the 22nd June 2021 that the first official authorisation to market edible insects within EU was granted to Agronutris, an European pioneer of insect production as protein source. The obtention of this authorisation is a historical and necessary milestone for the development of the edible insect market and the promotion of insect integration in the global dietary patterns to address environmental and health challenges. The authorisation obtained by Agronutris required the filing of a novel food dossier. In this presentation, the content of the application dossier will be detailed (production process, compositional data, toxicology, allergenicity). Then, the filing process will be described from the first step of required data identification to the obtention of the authorisation of marketing the Novel Food. Furthermore, the specificity of this first insect novel food will be highlighted in terms of labelling, conditions of use and data protection. Finally, the input of this first authorisation to the whole insect industry will be discussed focusing on the insect food products expected on the market within 5 years.

How the insect industry is maturing and adopting standard quality control measures

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While most other livestock industries have been around for centuries, large scale insect production has just barely wet its feet. The field of insect mass rearing began in the 50's and 60's with the mass production of sterile males and was expanded by the rearing of beneficial insects in the 70's, 80's and 90's. Its current main driver, however, is the mass production of insect biomass for feed and food. Because of this shift the main focus will be on the volume of insect biomass produced, rather than the number of individuals. Another important focal shift will be the quality assurance pertaining possible food safety issues with biological and chemical contaminants. In general, all quality control and assurance aspects, in the insect mass rearing industry has only began to take shape in the last couple of years. As this industry branch gains momentum, more and more players are joining the search for state of the art technology, standard operating procedures, legal framework and health and safety standards to supply the potential demand for insect biomass. The insect mass rearing industry for feed and food has reached a point where a few big players managed to launch their first industrial facility and literally thousands of other small and mid-scale companies are making their way to join them. Cogastro is a company that delivers farm management software for insect farms to help streamline standard operation protocols, production planning, documentation control, scheduling, data management and analysis and quality control. Therefore Cogastro is in a unique position to observe how insect production companies are dealing with their data and to see the evolution and maturation towards a well-established industry.

Insects as animal feed: industry's need for clear & defined policy

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The use of insects as a protein source or agricultural input is an exciting area, filled with potential for improving cost, efficiency, and our food system. These uses for insects range from human consumption, livestock feed, composting aids, and as organic matter in fertiliser. This array of useful options has created industry interest in Canada, and abroad, to widen the use of insects in mainstream agricultural practices. There is a great deal of information on insect protein itself, while policy surrounding the topic is clearly lagging behind industry needs and interests. The Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA) has identified this lack of policy as a gap in their promotion of better farming practices and agricultural wellbeing in the province. This research aimed to define existing global policy on insects as animal feed in an effort to inform OMAFRA's future policy on the matter. The lack of targeted and specific language in current global and domestic insect policy was detailed through the use of a jurisdictional scan on insect protein with a focus on agricultural applications. This policy gap fails to adequately address pressing issues, such as: Does producing insects and/or feed equate to being a farm and how does that relate to tax codes? Are current western insect feed and organic matter production practices considered to be ethical? What practices are considered acceptable in the use of insects in a farming circular economy? And many wider questions relating to approved animal feed.

OECD Workshop

The design and construction of a pilot plant for insect rearing and processing in Belgium

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Insects have the potential to form the basis of a new sustainable value chain and to play a key role in the transition to a biobased economy in Europe. Several companies recognise the potential of insects, but immediate application on an industrial scale is hampered by several uncertainties. In Belgium, KU Leuven, Thomas More and VITO have acquired expertise in rearing and processing of insects on laboratory scale, while in practice there is a clear need for expertise on pilot scale as an intermediate step towards industrial production. In 2018, the construction by KU Leuven of a versatile pilot plant (Geel) started and in September 2020 this plant was put into use by KU Leuven and Thomas More for rearing, harvesting and processing of insects. Currently, 4 insect species are being reared: *Hermetia illucens*, *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*. This plant is equipped with 3 climate chambers and 6 climate cabinets. Different rearing containers and systems provide the opportunity to investigate various rearing techniques for the mentioned, as well as for other insect species. The plant has a separate feed treatment and storage room. Through the harvesting room, collected insects can be transferred to a separate room where they can be killed and stabilised by i.a. blanching, steaming, oven and radio frequency drying. The pilot plant also has 2 research labs, one of which is equipped and licensed for the investigation of foodborne pathogens during the rearing and processing of insects. A bio-refinery park (Mol), including reaction vessels, decanter continuous centrifuge and membrane filtration units, offer the possibility to fractionate the insect biomass to tailor-made fractionation. Today both sites of the Insect Pilot Plant are used for multidisciplinary research and demonstration projects in which the developed expertise is the basis to support interested research partners, companies, the government and the general public. More info: www.insectpilotplant.be

Research methodologies to determine agronomic value of frass

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As society looks to technologies and practices which contribute to a circular food system, black soldier fly (BSF) larvae are emerging as an effective way to process food waste, which produces both larval biomass as feed and frass as a soil amendment. Research evaluating frass as a soil amendment is in its infancy, and existing research differs widely in methodology and quality. In defining best research practices to evaluate frass we needn't reinvent the wheel; there is much that we can learn from existing research evaluating other organic matter-based soil amendments like manure and compost. 'Agronomic value' of a soil amendment can encompass many properties which impact crop yield or quality, including nutrient provisioning, increase in soil organic matter, improvement in soil structure, adjustment of microbial community, and in the case of frass, perhaps even protection from fungal pathogens. Appropriate methodologies depend on which property we wish to evaluate and which system we want to evaluate them in (e.g. greenhouse production, market gardening, field cropping). Some properties (e.g. short-term nutrient provisioning, carbon cycling, or microbial community response), can be evaluated experimentally in one or two seasons, but many important properties (e.g. soil structure impacts, soil carbon accumulation) will require long term agronomic studies. Many would consider nutrient provisioning to be the most important component of frass' agronomic value. Experiments to evaluate provisioning of a given nutrient should consider, and if needed compensate for, deficiencies in other nutrients which may confound results. For example, a pot study evaluating phosphorus provisioning should usually supplement the soil with non-limiting nitrogen. Further, experiments should include relevant comparison treatments, including unamended soil at least one common organic or conventional fertiliser. Many best practices for frass evaluation can be borrowed from longstanding research in manure, compost, and other organic nutrients. To this end, it is vital that BSF researchers partner with agronomists and soil scientists with experience in soil amendment research.

Factors influencing incorporation of insect meals in animal studies

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Insect meals are considered as the most promising alternative feed sources. As a consequence, their use is still not a common practice and their inclusion requires the consideration of various factors to optimise growth, animal welfare and feed costs. The insect meal form (whole or defatted) directly impacts the level of inclusion, in particular in feeds where low amount of lipids is needed (i.e. poultry). From a nutritional point of view, the factors that influence the meal composition, besides the differences linked to the insect species, are the rearing substrates and production processes. Process (drying, defatting) can also dramatically influence the nutrient digestibility and availability that have to be assessed through *in vivo* or *in vitro* trials, with differences being observed in relation to the entity of the defatting process as well. Furthermore, the adoption of a full-fat or a defatted meal may also impact the quality of the final product (fatty acid profile). Low digestibility of chitin is also a limiting factor. Studies on the possibility of increasing the digestibility of insect meals using additives are ongoing. For these reasons, when different suppliers are used for feed production, chemical analyses need to be performed. In addition to the nutritional aspect, in some zootechnical species (i.e. fish), a physical evaluation of the feed is necessary. In particular, the viscosity effect that a whole larvae meal rich in fat confers to an extruder die can decrease pellet stability resulting in nutrient loss. Palatability is also important as it affects feed ingestion. Even though insect meals seem well accepted, some palatability issues have been reported at high levels of inclusion. It is however not clear if these issues were due to the level of inclusion or to some intrinsic characteristics of the meal used. Finally, the crucial factor for the future practical incorporation of insect meals in animal feeds is the availability and consistency of the supply. Without large and consistent quantities it will be difficult for feed producers to incorporate these alternative ingredients within their production processes.

The (im)possible standardisation of insect feed experiments: a BSF case study

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The repeatability and reproducibility of experiments is one of the core foundations of science. This implies that all necessary information is described and experimental procedures are standardised. That is the reason why there are countless protocols to ensure repeatable and reproducible results for chemical analysis, ecotoxicology, physics, etc. The field of insects for food and feed is young and the number of publications is booming. For example, there is a lot of interest in using the black soldier fly to convert low value biomass into high-quality feed. However, there are nearly as many experimental protocols as there are papers, which inhibits the direct comparison of the results. An international alliance was formed with partners from EAAP, SUSINCHAIN and OECD to discuss standardisation possibilities for the first time and create a feed experiment protocol. This protocol was validated by 9 partners for 3 feeds in a ring test. The whole process and the results of the ring test has been a real revelation. The results reveal the expected variation within an experiment for different parameters (e.g. growth and yield) and what to expect when comparing results among laboratories or publications. By combining this information with recently published information (e.g. the genetic diversity of the BSF) we now have a better understanding on what is possible, which parameters are important (or not). What can and will go wrong? What is critical information for any publication? What can be improved in the future?

Diseases of black soldier flies, research and diagnostic tools

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Pathogens are a challenge for all biological production systems. For producers of insects for food and feed, they can result in extensive production and economic losses. Black soldier flies (BSF) are considered to be very robust and resistant to insect diseases, however emerging diseases can appear in the future and reports about higher black soldier flies mortality are increasing. Insects can become infected by bacteria, fungi, microsporidia, nematodes and viruses and the main biological characteristics of those pathogens will be described. BSF have been experimentally infected by fungal, bacterial and nematode entomopathogens in the laboratory and recent bioinformatics studies have revealed evidence of the presence of viruses in BSF samples. For insect diseases, except for honeybees, there remains a lack of diagnostic procedures and certified laboratories. For certain insect diseases like some known fungal and bacterial diseases such diagnostic procedures could relatively easily be developed, whereas for other less studied or emerging BSF pathogens, this will require further research and development that include collaboration between scientists, laboratories specialised in insect pathology as well as with the industry..

Standardisation of research methodologies: the example of best research practices established for honeybees

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Honeybees play an important role in modern agriculture as farm animals and crop pollinators, and they are responsible for a third of our diet. Over the last few decades, managed honeybees have been facing large-scale losses worldwide. There are various causes, including the spread of pathogens and parasites, habitat loss and loss of forage spaces, the use of pesticides, and the occurrence of climate changes. Many scientists investigated these issues from all over the world separately and independently, and often using different methodologies. This approach might lead to conflicting and sometimes erroneous findings. To provide global and sustainable solutions, a group of bee scientists, established COLOSS, a non-profit association for the prevention of honeybee Colony LOSSes. The mission is to investigate both the causes of the declining bee health as well as effective means to improve the well-being of bees. COLOSS endorses core projects and tasks forces focusing on specific topics identified by the association to receive priority attention. Among the Core projects, the COLOSS BEEBOOK is a unique venture that aims to standardise methods for studying the honeybee. The vision of the project has been to develop a definitive inventory of standard techniques and methods in honeybee research, to ensure that studies performed by different laboratories around the world would be directly comparable with each other. It is a practical manual compiling more than 2,000 standard methods in all fields of research. It is definitive, but evolving, research manual. Chapters describe methods for studying honeybee biology, methods for understanding honeybee pests and pathogens, methods for breeding honeybees, and methods for understanding hive products, including for quality control purposes. The COLOSS BEEBOOK is a testimony to the accomplishments made when scientists work together with a common purpose and can inspire the development of standard methods in mass-reared insect research.

Evaluation of the antifungal activity of black soldier fly larval frass using the double-layer agar technique

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Black soldier fly larvae (BSFL) have the potential to efficiently transform a wide range of organic waste into larval biomass with a high nutritional value. A secondary product obtained from this process is frass, which includes larval excrement, larval exoskeleton, and undigested diet. Increased research on frass is needed for its valorisation on an industrial scale and in the circular economy. We have reported that frass possesses antifungal activity against phytopathogens based on the antagonistic effect between the frass microbial community and phytopathogens. Our initial work employed conventional approaches to study antifungal activity based on competition between antagonists and pathogens for food and space. However, antibiosis which is the suppressiveness of phytopathogens by antimicrobial compounds produced by fungi or bacteria requires an alternative assay. The double-layer agar assay is a well-established technique to test microorganisms for their ability to produce antimicrobial compounds. We evaluated the antifungal activity of a wide range of water-based frass extracts against several fungal/oomycetes phytopathogens including *Pythium ultimum*, *Rhizoctonia solani*, *Phytophthora capsici*, *Fusarium oxysporum*, *Alternaria solani*, *Sclerotinia sclerotiorum*, and *Botrytis cinerea*. The inhibitory effects of metabolites produced by the frass microbial community on mycelial growth of every pathogen tested, as evidenced through the double-layer agar assay, reveal strong antifungal activity against *B. cinerea*, *A. solani*, *P. ultimum*, *R. solani*, *P. capsici*, *F. oxysporum* and *S. sclerotiorum*. The double-layer assay provides a discrete and sensitive approach to assess *in vitro* antifungal activity of frass against a range of important phytopathogens.

Environmental conditions influencing growth and development of black soldier fly (L.) (Diptera: Stratiomyidae) larvae

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The growth and development of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae) has been studied in depth for nearly three decades. From these studies, we have learned that numerous abiotic parameters such as temperature, humidity, and light can have drastic and varying impacts on the production of the black soldier fly population in colony. This presentation will provide a review of the literature on studies of the impacts of abiotic parameters on black soldier fly growth, with a focus on the commonalities, differences, and industrial implication of these studies. Additionally, recommendations to help standardise and broaden the impact of future research studies will be made.

Feeding studies in insects: what are we measuring?

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Interpreting feeding studies requires an understanding of what animals consumes and how they utilise this consumed material. This can be approached via 4 questions: (1) what is in the feed; (2) what is consumed; (3) which parts of the feed are digested and absorbed; and (4) what are the absorbed nutrients used for? The first question can be approached by chemical analysis of the feed. Chemicals of interest need to be defined and suitable analysis needs to be determined and executed in accordance with standard methods. For insects with moist diets, analytical outcomes are influenced by microbiota. Microbiota interfere in several ways; compete for feed, provide nutrients and other components via their metabolism, or serve as a source of feed if digested. The second question, what is consumed, seems straightforward. However, there are certain species-specific issues. For example, yellow mealworms, which live within their substrate mix faeces with feed. Moreover, they can consume this feed-faeces mixture more than once, complicating accurate quantification of feed intake. Besides, are all ingredients consumed equally, or is there selection? The third question, which parts of the feed are digested and absorbed, is barely touched upon in insect studies. Most studies focus on apparent faecal digestibility, whereas e.g. true ileal amino acid digestibility, is not considered. Should it be? The last question, what are the absorbed nutrients used for, relates to which tissues are synthesised. Currently, total weight and total composition are considered, but perhaps analogous to conventional livestock this should be refined. Answering these questions requires correct sampling. This includes ensuring a sample is representative for a batch, and that the sampling does not disturb the rearing process, if later samples are to be taken. In practice, there are inherent trade-offs regarding accuracy, and time and budget investments. To summarise, this talk will provide a short overview of relevant aspects in feeding studies, focusing on the pitfalls associated with nutritional trials for insects.

Health and biosecurity

Biosecurity, workers health issues and the insect production enemies: a growing concern

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With the increase in the number of insect producers and the consequent expansion of breeding, the young entomophagy industry now faces growing biosecurity issues. Biosecurity is defined as a set of measures aimed at reducing the risks of transmission of pathogens or harmful outputs for the environment, workers, livestock and consumers. Insect farms form large populations of a single species, which can include hundreds of millions of individuals in the same place. This concentration increases the risk of spreading diseases. In addition, farms relying on the recovery of organic residues (entotechnologies) are more at risk of infiltration of opportunistic organisms in farm inputs and outputs. To limit bacterial, viral, fungal or entomological risks, it is essential that breeders implement preventive measures, such as health safety, biocontainment and genetic diversification protocols. There is a growing need for more research on probiotics, functional food additives and immune priming in order to avoid potential antibiotic resistance in farmed insects. Moreover, in light of new surveys conducted in 2021, strict health and safety protocols remain of paramount importance to protect workers against the risk of infection, sensitisation or allergies. Another survey result highlighted the end product innocuity as a prime public concern for the acceptability of insect in food.

Black soldier fly frass with antagonistic biocompounds as a potential biofungicide against *Fusarium oxysporum*

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Phytopathogenic fungus *Fusarium oxysporum* (FO) strains are among the most devastating fungal plant pathogens globally, affecting 100,000 ha of cropland and causing \$2.3 billion USD of losses in the last century. Of particular concern is *F. oxysporum* f. sp. *cubense* Tropical race 4 (Foc TR4) which has caused disease outbreaks so severe that it is considered an existential threat to the banana industry. As of this writing, there are no effective chemical or biochemical agents that can be used to manage Foc TR4. Here, we present preliminary evidence that insect frass inhibits FO growth, including Foc TR4, indicating its potential use as a biofungicide. We reared black soldier fly (BSF, *Hermetia illucens*) on organic waste-streams to generate frass which was subsequently used to prepare aqueous extracts. In extract preparation, we investigated the effect of nutrient additives and aeration on inhibitory activity. Our *in vitro* studies demonstrate that BSF frass extracts cause a 90% inhibition of FO mycelial growth. In the case of Foc TR4, the frass extracts achieved a 77% inhibition compared to 0% for controls consisting of soil and a commercial fertiliser. In addition to antifungal activity, it was tested against FO using a disk diffusion method. The frass samples contain feed residues, exuviae which contain chitin, antimicrobial peptides (AMPs), and microbial colonies. Thus the anti-phytopathogenic activity could be due to the antagonistic substances produced by the microbes such as the AMPs, enzymes and/or other biocompounds with antifungal activities. Additionally we investigated the presence of siderophores, chitinase, lipases and proteases in BSF frass. We demonstrate the potential of BSF frass as a promising resource to control the economically significant phytopathogenic fungi FO strains.

Insights into the immune system of black soldier fly

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The innate immune response of insects provides a robust line of defence against pathogenic microbes and parasites. It consists of cellular and humoral mechanisms that are conserved across insect species, however peculiarities on the timing and the efficacy of triggered immune responses, as well as on the haemocyte populations that are involved in the defence response, have been reported. In this scenario, an insect that deserves particular attention is the black soldier fly, *Hermetia illucens*, which is widely used for the reduction and valorisation of waste and by-products. Black soldier fly larvae are generally grown on decaying organic substrates that can bring the larvae into contact with potential pathogens, challenging their health status and growth with dramatic effects on the rearing system. Thus, information on the mechanisms that allow this insect to counteract these threats is needed. In the present study, we performed a morphofunctional characterisation of haemocytes in *H. illucens* larvae, investigated the processes underpinning the immune response to bacteria, and analysed their reaction times. Our data demonstrate that at least six haemocyte types take part to a finely regulated cellular response, to counteract the diffusion of foreign agents in the insect body. Moreover, while phagocytosis and encapsulation are rapidly activated after the immune challenge, the humoral components intervene later. Although this synergic action of the two branches of the immune system is able to completely remove Gram-positive and Gram-negative bacteria from the body within a few hours after injection, Gram-positive bacteria persist in the haemolymph longer than do Gram-negative bacteria. Finally, the activity of lysozyme and phenoloxidase, two key players of the humoral response, show unusual dynamics. This study represents a baseline of knowledge for future studies on the immune response of larvae challenged with other infectious agents and is a prerequisite to manipulating the larval immune response by nutritional or environmental factors to increase insect resistance to pathogens. The study was funded by Fondazione Cariplo (2020-0900).

Exploring the plasticity of the gut microbiota in the larvae of *Hermetia illucens*

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The black soldier fly (BSF, *Hermetia illucens*) has become one of the most common mass-reared species in the world for the production of insect protein for animal feed. This evolution also drives a need for more research on BSF to optimise its efficiency and sustainability on various diets. One area that is being explored is its gut microbiota. Indeed, microbes in the gastrointestinal tract of traditional livestock, and also insects, exert important functions to modulate animal health and performance, e.g. the processing of dietary components. Given the importance of these interactions between the host and its microbiota and their influence on host phenotype, it is crucial to understand: (1) how the composition of the microbiota varies in response to various biotic and abiotic factors; (2) if there is an 'optimal' microbiome from a production perspective; and (3) how the microbiota can be manipulated to achieve this optimum. In the frame of an ongoing project funded by the Research Foundation – Flanders, our research group (IP&P) explored how a number of biotic and abiotic factors impact the composition of the microbiota: (1) time (consecutive rearing cycles); (2) space (different gut sections); (3) genetic diversity (different BSF genotypes); and (4) co-existing virome (bacteriophage community can impact the microbiota via predation). The factor time was assessed by sampling six consecutive rearing cycles at two locations (inter-cycle) at two time points in each cycle (intra-cycle). For space, four different gut sections (anterior/middle/posterior midgut and hindgut) were sampled on three substrates (chicken feed, DDGS and a food waste mixture). Genetic diversity was investigated by sampling larvae from four different genotypes on three diets (chicken feed, a food waste mixture and chicken manure). The microbial community composition was always determined using 16S rDNA sequence analysis. Finally, the co-existing virome was determined using an adaptation of the NetoVIR protocol. Here, we summarise our findings and discuss our improved global understanding of the plasticity of the microbiota in the BSF larval gut.

Effect of density and fungal co-stressing on the viral abundance of the *Acheta domesticus* densovirus in house crickets

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The house cricket *Acheta domesticus* is a promising candidate for food and feed purposes, but pathogens can cause epizootics in its rearing. Crickets are usually reared in high densities to maximise cost effectiveness, but this creates a highly favourable environment for disease outbreaks. The *A. domesticus* densovirus (AdDV) is one of the major pathogens that can cause serious losses in production of house crickets. The virus is present in most *A. domesticus* stocks in a covert state, but can become overt. Co-infection with other bacterial and fungal entomopathogens and abiotic stresses are suggested to be the triggers for transition to the overt state of AdDV. Rearing density is hypothesised to be an important factor for AdDV outbreaks, but the correlation between rearing density and AdDV titers has yet to be confirmed. To explore this we designed an experimental setup with three different rearing densities, combined with exposure to two different fungal pathogens. We recorded the effect of co-stressing on the mortality and weight gain of individuals and measured AdDV viral abundance on group level using qPCR. High rearing density and fungal pathogens reduced survival rate, but did not affect average end weight. There was no interaction factor between the two stressors in case of survival rate or weight. Results on the effect of the two stressors on AdDV load are currently being analysed and will be presented. Producers need to consider chances of higher mortality with higher densities, but the losses due to mortality might be outweighed by the higher weight output from the same surface area. An optimal rearing density leading to high productivity while keeping the risk of collapse of the production batch minimal needs to be established.

Virus exposure bioassays to assess uptake and survival of a coronavirus in *T. molitor* and *H. illucens* larvae

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The mass production of edible insects offers the potential for a more sustainable source of nutrients for livestock feed. In order to ensure this sustainability, there is a need to explore new substrates for the insects, including those of animal origin, such as slaughter by-products, manure and communal waste. However, such substrates could pose risks that need to be assessed, such as the transmission of viruses. Experimental exposure studies of insects that are used for feed and food with animal viruses can provide more knowledge about such hazards for use in future risk assessments. In a series of experimental studies, we exposed two insect species to a porcine respiratory coronavirus (PRCV). This virus was used as a first model for other viruses of interest. For virus exposure of *Tenebrio molitor* larvae, a newly developed virus exposure bioassay, using oral inoculation, was established. For virus exposure of *Hermetia illucens* larvae, a method for inoculation via feed spiked with PRCV was tested. Both species of larvae were sampled immediately following virus exposure and until 8-9 days post inoculation. The presence of viral RNA was determined using quantitative RT-qPCR and that of infectious virus using virus isolation in cell culture (ST117 cells). PRCV RNA was detected in *T. molitor* on day 0 and for up to 3 days following virus exposure, and in *H. illucens* on day 0 and for up to 2 days. The amounts of viral RNA were low in the samples after day 0. Using virus isolation assays in ST117 cells on samples with the highest amount of viral RNA, infectious PRCV was detected in one *T. molitor* sampled at 0 days post exposure. While it can be concluded that PRCV does not survive well in the fed insect larvae, and that the bioassay developed for *T. molitor* larvae was adequate, there remains some work on optimising the *H. illucens* bioassay. Future work will therefore focus on the latter and on new exposure bioassays using the problematic African swine fever virus.

Evaluation of the impact of the parasite *Tribolium castaneum* on the mass rearing of *Tenebrio molitor*

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The use of insects is considered a promising alternative to protein sources for animal and human food. The mealworm *Tenebrio molitor* is one of the most widely used insects on an industrial scale for this purpose. However, insect farming is subjected to constraints, especially, the predation by other insects. The objective of this study was to estimate the rate of parasitism of *Tribolium castaneum* (Tr), a small beetle pest often found in stored food stuffs like wheat bran, the basic food substrate of *T. molitor*. The presence of this pest is a serious danger to mealworms. The effect of six densities of adults of *T. castaneum* (0.00, 0.09, 0.18, 0.36, 0.72 and 1.45 Tr/cm²) was evaluated on the rearing performance of *T. molitor* under laboratory conditions (T=28±1 °C, RH=70±5% and total darkness). The experiment was run in small cylindrical plastic boxes (section=22 cm²; H=6 cm) filled with 12 g of wheat bran (5 repetitions) and containing 18 adults mealworm. The parameters were evaluated after 40 day. The fecundity of *T. molitor* was significantly affected by the presence of *Tribolium*. A decrease of 42% in larval production of *T. molitor* was observed when density reached 0.72 Tr/cm². Mealworm larval biomass produced per box was significantly affected by *Tribolium* density. Without *Tribolium*, the mealworm larval biomass reached 11.77±0.69 g, however it reached only 5.09±0.66 g in the box containing 2.66 Tr/g of wheat bran. Increased pest density also impacted negatively the conversion rate. However, it did not impact the average weight of mealworm larva. We recommend to use wheat bran containing less than 0.18 Tr/cm² or 0.33 Tr/g of wheat bran to maintain an optimal mass rearing of *T. molitor*.

***Hermetia illucens* prokaryotic and eukaryotic microbiota composition and interactions at different life stages**

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The black soldier fly (*Hermetia illucens*) larva feed and grow on organic waste containing high density of microorganisms and potential pathogens. As the economical and ecological impact of black soldier fly rearing gains increased importance, understanding how the holobiont composition and interactions affect performance and health of the host becomes vital to the optimisation and specialisation of industrial scale rearing. The feed substrate is a known determinant of the microbiota's composition; however, the impact of life stages, interaction networks and vertical transmission have been neglected. This investigative study characterises *H. illucens* associated prokaryotic and, for the first time, whole eukaryotic microbiota's taxonomic and functional profile across the entire life cycle (eggs, larvae, prepupae and adults, from parent X0 to mature progeny X1) when reared in two substrates: (1) plant-based (housefly Gainesville diet); and (2) animal-based (poultry hatchery waste) (n=9). We developed a 18S blocking primer specific to the black soldier fly, enabling us to use a metataxonomic approach with target 16S and 18S genetic markers amplicon sequencing. As expected, microbiota is modulated by the feed substrate, but its composition and dynamics are also influenced by the developmental stage and time. Progeny switched to a different substrate had a major shift in the composition of their microbiota compared to parents. The eukaryotic microbiota was found to contain multiple ubiquitous opportunistic pathogens that should be considered in future biorisk management. We found that interaction networks in microbiota are linked to composition and developmental stages. Our study is a preliminary step toward the development of an artificial microbiota probiotic supplement to enhance *H. illucens* health and performance.

The role of the gut microbiota in host fitness and resistance to bacterial and fungal pathogens in wax moth larvae

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One great challenge of the future is securing enough food for a growing global population in a changing world. Fortunately, insects have been recognised as a more sustainable source of protein; however, our knowledge of maintaining insect health in a mass-rearing environment is lacking. In recent years, the gut microbiota has been identified as playing a critical role in improving host response to disease-causing pathogens, but the extent to which the microbiota contributes to the immune response is not fully understood. To explore the partnership between the gut microbiota and the immune response, *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae were reared in sterile (=axenic) and non-sterile (=conventional) conditions. Additionally, pathogenic bacteria *Bacillus thuringiensis* (Bt), and fungi *Metarhizium anisopliae* were utilised to assess the role of the gut microbiota via two routes of infection. Our results indicated that the conventional larvae had a greater resistance to a spore-crystal suspension of Bt after oral infection, with the surviving larvae being able to completely clear the bacteria from the gut over 96 hours. However, for the axenic larvae, 100% mortality was reached by 96 hours as compared to 50% mortality for conventional larvae. We are currently characterising the microbiota via 16S rRNA and Illumina sequencing during Bt and fungal infection, and at the same time analysing the expression of several important immune response genes using qRT-PCR techniques. Preliminary results show there is greater basal expression of immune response genes in conventional larvae resulting in the larvae being better 'primed' for pathogen challenge. Furthermore, phenoloxidase levels in the haemolymph of conventional larvae was greater in control conditions as well as post bacterial and fungal infection than the axenic larvae. As a whole, our results provide new insights in the understanding of how and to what extent the immune response is mediated by the microbiota, which can improve the potential for the maintenance of insect health.

A new mite capturing technology using electrocharged material, a trial challenge on insect feed and food production

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Ectoparasites cause one significant health and environmental concern in the urban environments on animal production fields. *Dermanyssus gallinae*, known as the 'red mite', is a hematophagous ectoparasite commonly found in laying hens and is one of the most important epidemiological and economic problems. Red mite infection induces a decrease in egg production poor egg quality and can ultimately lead to the premature death of hens from substantial blood loss. Additionally, the red mite is a candidate transporter of arboviruses. So an increase in the number of red mites may lead to a higher outbreak risk. Furthermore, the most effective timing for poultry red mites' spread environmentally around poultry farms is unknown and demanding control applications and the most effective extermination method. We have developed an electrostatically charged device (i-Trap[®]), that can physically attract and capture red mites and tangle and catch on them. This device has an electrical charge from static electricity created by the material's polyurethane composition. The i-Trap attracts and traps the red mites. In this presentation, to quantify the ectoparasite infestation population from which the contamination level can also be determined in insect feed production and used for basic science. In this study, we would like to propose the possibility of using this technology, patented in Japan, the U.S., China, and Thailand, in insect production. We want to introduce an option of contributing to insect feed production in our research by using the behavioural characteristics of mites in the capture technology and the possibility of using this technology to control external parasite contamination in the insect production area.

Effects of probiotics on *Tenebrio molitor* development and resistance against the fungal pathogen *Metarhizium brunneum*

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As the interest in the mass production of insects for food and feed increases, so does the need to ensure that the insects remain healthy in the face of pathogen challenges. This study follows from previous published work testing the effects of an indigenous *Pediococcus pentosaceus* strain KVLB19-01 on *Tenebrio molitor* larvae growth and survival. Here, we used the same methods to test two commercially available probiotic strains used for other livestock, *Enterococcus faecium* 'M74' and *Bacillus subtilis* 'King', on *T. molitor*. Additionally we conducted a pathogen challenge experiment using an entomopathogenic fungus, *Metarhizium brunneum* strain KVL 12-37, originally isolated from *T. molitor*, to assess the effects of the probiotics on the insects' immunity. The pathogen exposure was conducted on larvae reared on diets supplemented with the three different probiotic strains and a non-supplemented control diet in 9 weeks. 25 larvae from each of the 4 treatments we exposed to 2 µl fungal suspension (1.22×10^5 spores) and 25 larvae from each of the four treatments were exposed to 2 µl triton X (control) on the thorax. Results showed that two of the probiotic strains, *P. pentosaceus* and *E. faecium*, but not *B. subtilis*, improved larval growth rate compared to non-supplemented control diet. The mortality of the fungal exposed larvae were higher than the unexposed larvae reared on the same diet, but lowest in the larvae supplemented with *P. pentosaceus* and *E. faecium*. This study confirmed that dietary supplementation of probiotics in *T. molitor* larvae may add nutritional value to sub-optimal diet and boost insects' immune responses against entomopathogenic organisms.

Insect health in production systems: diagnosis, prevention and management

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All biological production systems are challenged by pest and pathogens and with the rapid growth of insect production for food and feed; diagnosis, management and prevention of existing and emerging insect diseases is urgently needed. Insect pathogens such as bacteria, fungi and viruses have the potential to develop into epidemics and in certain cases they can cause great economic losses to the producer. We will introduce important insect diseases known to occur in some of the main production insects and their biological characteristics. Furthermore, we will discuss management and prevention actions to promote insect health. Most life stock production systems have diagnostic procedures and certified laboratories, such facility has not yet been established for insect diseases. To facilitate network collaboration between scientist and laboratories specialised in insect pathology is needed.

Entomopathology and veterinary diagnostics: the old, the new, the borrowed and... the blue?

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Commercial insect production for entomophagy is a relatively young industry, with limited supporting resources, especially when it comes down to diagnostic and biosecurity counselling. However, there are other industries, such as production animal farming and beekeeping, that hold a longer history and established practices that can provide experience and knowledge to inspire best practices for insect productions. In animal productions, veterinary medicine is a crucial service to maintain not only herd health, but also to ensure that the food produced is safe for human consumption. Old and modern diagnostic tools (bacterial and fungal culture, Maldi-Tof bacterial identification, PCR, genomic marker-based measures of inbreeding, etc.) can easily be applied to insect productions, but interpretation of the findings can be difficult. Veterinary pathology can provide some of these answers: it is a cornerstone in animal diagnostics, as it provides insights on causative processes and agents. Although insects hold particularities that can complicate the application of tools and services available in other species, solving these issues should not be too big a task for the producers, diagnosticians and researchers that wish to devote time to this field.

Utilisation of yeast supplement for improving *Hermetia illucens* reproductive and larval performance

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Hermetia illucens, black soldier fly larvae (BSFL) can bioconvert a vast range of organic waste streams into high quality protein and lipids, suitable to be used as food and feed. However, the reproductive and performances can be highly influenced by the larval feed source. The study was conducted to assess the utilisation of four different Lallemand Bio-Ingredients' yeast products as supplement in BSFL production. The yeast products were: Torula inactivated yeast (TIY), B-vitamins fortified inactivated yeast (BIY), Mineral (selenium) enriched yeast (MIY) and Sterol inactivated yeast (SIY). A number of key performance indicators in *H. illucens* production (e.g. larval weight and survival rate, egg production) were assessed for the BSFL reared on a standard Gainesville diet (GD), made of alfalfa, wheat bran and maize. It was supplemented with either TIY, BIY, MIY or SIY at 5 and 10% inclusion rates. There were significant differences on larval performance between larval reared on the yeast and the control GD. The biggest larvae were obtained when the GD was enriched with 10% of SIY and TIY with 29 and 35% weight increase at harvest, respectively. Moreover, the utilisation of yeast as supplement to GD resulted in bigger prepupae larvae when compared with the plain Gainesville treatment. The survival rate was found to vary between 69 and 85%. Except for TIY 5%, the yeast inclusion treatments were found similar or higher survival rates than the control. These results indicate that the utilisation of Lallemand's yeast products as supplement in *H. illucens* rearing can increase the larval performance without affecting survival rate. The egg production was found to increase when the GD was enriched with yeast products, except for MIY 10%. Moreover, the highest egg production was obtained as a result of utilising TIY and BIY yeast at 10% inclusion rates. These results indicate that the inclusion of Lallemand's yeast products as feed supplement in *H. illucens* production could increase egg production.

Growth performance of yellow mealworm larvae (*Tenebrio molitor*) fed on wheat bran supplemented with natural botanicals

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During the last decade, insects have received increasing interest as innovative sources of alternative protein for food and animal feed. Yellow mealworm (*Tenebrio molitor*), is considered one of the best options, rich in protein, easy to breed and feed. In a mass rearing context, the improvement of the growth performance of the yellow mealworm larvae is a key issue. Thus, the aim of this study was to compare the growth performance of yellow mealworm larvae fed on wheat bran supplemented with eight (8) botanical ingredients. More exactly, 3 classes of botanicals were tested at various inclusion levels, i.e. essential oils (0.5, 5, 50 mg/kg), plant extracts (1, 10, 100 mg/kg) and crude dry powders (5, 50, 500 mg/kg). Each additive was added at 0.1% of the wheat bran control diet and tested in 3 replicates. The measurement of the growth performance of each rearing crate was performed on a weekly basis until the 5th week corresponding to the harvest of larvae. Data clearly showed a significant growth improvement of yellow mealworm larvae with the crude plant technology (final average weight of 116 mg; $P < 0.05$) whereas purified substances did not show significant results compared to the control diet (final average weight of 98 mg; $P > 0.05$). Based on these preliminary data, synergetic effect of combined crude dry powders will be evaluated on growth performance and potentially on larval nutritional values as well. In conclusion, this study highlighted the great potential of the use of botanicals as a dietary supplement in mass rearing of yellow mealworm larvae.

Characterisation of compounds active against *Aeromonas hydrophila* isolated from the gut microbiome of *Hermetia illucens*

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The larva of *Hermetia illucens* has a gut microbiome that plays an important role in the digestion of waste and prevents the colonisation of pathogenic microorganisms. The aim of the study is to identify potential sources of new antibiotics from fungi present in the gut of *H. illucens* larvae that are active against the bacterium *Aeromonas hydrophila*, which is responsible for economic losses in aquaculture. *Aspergillus* sp., *Scopulariopsis brevicaulis*, *Chrysosporium multifidum* and *Penicillium janthinellum* fungi isolated from *H. illucens* larvae were tested against *A. hydrophila* ATCC 7796 using the coculture method. As a result, the Antimicrobial Potential values of 1,6; 1,5; 1,3 and 1,5 were generated from the growth inhibition diameters, respectively. Subsequently, extracts obtained from the culture broths of the most active fungus were evaluated by bioautography, and the half inhibitory concentration (IC₅₀) and minimum inhibitory concentration (MIC) were also determined. The dichloromethane (DCM) extract of *S. brevicaulis* showed an IC₅₀ of 0.3 mg/ml and a MIC of 0.5 mg/ml against *A. hydrophila*. This DCM extract was fractionated by chromatography and the microbiological activity and the profile of compounds present in these fractions were analysed by ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-HRMS). As a result, 11 fractions were obtained. Among them, two fractions exhibited the best activity by bioautography. In the preliminary analysis by UPLC-HRMS some compounds were annotated by comparison with databases, among them lipopeptides and derivatives of hiocholic acid. Bioguided purification assays are in progress to characterise the compounds responsible for the activity against *A. hydrophila*. Our investigations suggested that the larva gut-associated microbes are able to antagonise the zoonotic pathogens.

Impact of a bacterial-based biocontrol product on zootechnical performances of *Tenebrio molitor*

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During the last decade, insects have received increasing interest as an innovative source of alternative protein for food and animal feed. Yellow mealworm (*Tenebrio molitor*), is considered to be one of the best options, rich in protein, easy to breed and feed. In a mass rearing context, three main objectives could be considered to ensure good performance: (1) reduce the mortality of insects; (2) enhance the laying rate of beetles; and (3) increase the growth performance of larvae. Thus, this study aimed to assess the impact of Noliflore Insects, a biocontrol product containing *Bacillus subtilis* strains, on zootechnical performances of *T. molitor* rearing: from the reproduction phase to the end of the growing phase. Two treatments were compared: a control group fed on wheat bran and a test group fed on wheat bran supplemented with Noliflore Insects. During the reproduction phase, the product didn't show any impact on breeding adults mortality, neither on laying rate. By contrast during the growing phase, the Noliflore Insects supplementation showed a significant reduction of the larval mortality and a lighter weight for the test group compared to the control. Based on these preliminary data, the correlation between insect densities and larval weight is being assessed. Besides, the impact of this biocontrol product on the pupation efficiency as well as on zootechnical performances of the second generation of insects remains to be evaluated. In conclusion, this study provides a good insight of the potential of the use of Noliflore Insects in the biocontrol of *T. molitor* production systems to reduce larval mortality rate.

Networking for the Edible Insect Sector / Other

Implementing a research network on edible insects to better understand the value chains

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We have a good knowledge about the edible insects species consumed by man. Most of the edible insects are gathered in the wild. But the value chains and the impact of the extraction of the insects on the wild population of the concerned species are not well documented. The GDRI Insectinov-Sud bring together laboratories, researchers, from fields like entomology, food sciences, anthropology and sociology and expert members of partner associations and NGO's who are concerned with the analysis of value chains on edible insects and the development of breeding with a view to their mass production and their transformation into products available for human and animal consumption. It constitutes an 'exchange forum' between these partners to promote the emergence of research and development projects and training of students through field schools. Amongst the results already obtained, we present the results of a study of the 'Edible caterpillars' value chain where the collect, the commercialisation and the consumption of caterpillars have been described in RCA. The quantities of caterpillars collected each year raises questions about the sustainability of the market especially considering the global changes, which affect the region. Rearing insects would be a part of the response and the African partners have built up a pilot farm producing Plan Weevils in Cameroon. The preliminary results of two projects on the edible insects market in Latin America will be presented. Rearing edible caterpillars would be another challenge. Interdisciplinary and participative research brought valuable insights in the Edible insects consumption and production.

Insects, art and advocacy – using visual art to cultivate interest in edible insects

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I am a visual artist best known for creating elaborate installations in which real insects, albeit dead and dried, are pinned directly to the wall in order to create patterns which reference wallpaper. When viewers enter one of my installations, they are greeted with something they think they know, that is, a patterned wallpaper. However upon closer examination, one discovers that it is entirely made up of insects. Emotions range and rapidly fluctuate from fear to awe to distaste to wonder. There is beauty in the pattern, yet the apprehension we feel toward insects creates a tension. My work seeks to provoke a series of increasingly urgent questions in the age of climate change and argue that beyond clean air and water, insects are the key to the earth's well-being and our very survival. Continued existence may well include the consumption of edible insects, however persuading people in North America to eat them is a challenge. A recent work entitled the 'Animal Dinner Party' is based on the song 'I Know an Old Lady Who Swallowed a Fly.' To rid herself of the fly, she swallows a spider, a bird, a cat, a dog, a goat, a horse and finally a cow, and then unsurprisingly she dies. The old lady's response to the fly exemplifies the fear many people have of insects. At my dinner party, animals (taxidermy) were seated at a dining table in a room adorned with insect 'wallpaper.' They appeared to be in lively discussion and enjoying a repast of bread, honey, and flies. The work provides multiple opportunities to discuss issues related to edible insects and food security as well as many of the most pressing issues of our time including systemic racism. Wouldn't it be wonderful if the world's problems might be solved over a meal? At other exhibition venues, I have painted gallery walls a reddish magenta type colour with a cochineal based dye/paint mixture. Cochineal is a scale insect from which a common food colorant is derived. A few years ago outrage was expressed when it was learned that Starbucks' used it to colour a smoothie. Was the anger over the fact that the drink was not vegan or that it had 'bug juice' in it? My presentation will explore the ability of art to cultivate discourse on the importance of insects and in particular those that are edible.

Going global with GRIA – a 'global roundtable for insect agriculture': representation, collaboration and best practices

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Insect industry growth, investment, competitiveness improvement, competition, cooperation and publicity continue to make progress for the sector, with diverse and ongoing milestones achieved. Regional collaborations are increasing insect sector internationalisation, inclusivity and influence. The collaboration between insect associations has continued to intensify over the last few years. Since 2019, NACIA, IPAA, IPIFF and AFFIA have met to accelerate this and work together on developing unifying themes to enhance moves for overall sector development. Internationally climate change awareness escalates, SDG deliverable targets rapidly approach and massive food system changes are seen as critical to meet food system challenges as part of initiatives like Scaling Up Nutrition, Feed the Future, Farm to Fork, and the UN Food Systems Summit. Additional needs are also becoming apparent as food security crises emerge in many locations. The global challenges facing food systems make collaboration between stakeholders from all parts of the food value chain critical. Many of these factors are entirely relevant for sub-sectors such as the edible insect sectors for both food and feed, and the diversity of business types, scales and issues faced make collaboration across different regional sectors important. The Global Roundtable for Insect Agriculture's vision is to provide a unified voice and leadership to represent and promote the global insect industry as an essential participant in the feed, food, fertiliser, and sustainable manufacturing industries. Three key goals are to: (1) provide a strong industry platform for representation; (2) strengthen collaboration internationally; (3) develop best practices. Highlighted will be both the 'Why?' and also specific features of the collaboration – such as the development and content of a joint OGSM document (objectives, goals, strategies, measures), work on FAO Codex alignment and a sector White Paper, and processes for increasing inclusivity amongst additional regional groups to meet the expectations set by the group's name: the Global Roundtable for Insect Agriculture.

Quebec edible insect industry portrait

J. Larouche

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In Quebec, the edible insect industry is rapidly expanding with several new producers and processors establishing themselves every year. According to the Vitrine Entotechnologique, 19 new insect producers were in activity between 2016 and 2020. However, the emerging industry is facing several challenges such as regulation, process optimisation, supply, social acceptability, training of qualified workforce and access to markets. In Quebec, 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 optimised industry development. To ensure the growth and sustainability of this young industry, the major players joined forces in 2021 to create the Table Filière des Insectes Comestibles (TFIC). This concertation table regroups 30 actors from all sectors such as waste management, insect production, insect processing, distribution, research, technology transfer, education, outreach organisation and government. Considering the sector's rapid growth, an updated portrait of the edible insect industry in Quebec is necessary to allow the documentation of the sector's economic perspective. Furthermore, the needs of insect producers and processors in Quebec will be assessed in terms of support, regulation assistance, research, and more. One of TFIC's other activities is to form focus groups to assess the best plan of action to answer specific and critical industry challenges. Indeed, a frass focus group has been assembled to better understand the current legislation which applies to frass, to define frass in a legislative point of view and identify best quality criteria for frass analysis. At the same time, we are also collaborating with the International Platform of Insects as Food and Feed (IPIFF) and the North American Coalition for Insect Agriculture (NACIA) to adapt the Guide on Good Hygiene Practices to the North American context. TFIC also has plans to address the many other initiatives currently taking place throughout the insect industry. This concertation initiative will contribute to the sustainable growth and perennity of the edible insect sector in Quebec.

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