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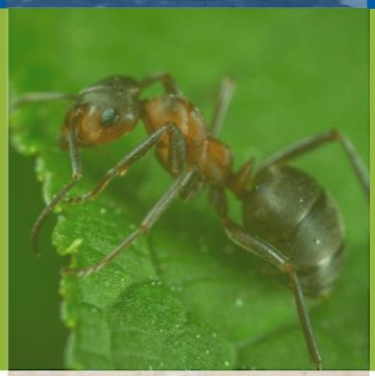
Bornimer Agrartechnische Berichte

INSECTA 2023

International Conference



Book of Abstracts

13th and 14th September 2023
Magdeburg, Germany

INSECTA

ATB

Bornimer Agrartechnische Berichte
Heft 107
Potsdam 2023

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Preface

INSECTA 2023 conference is organized on September 13 and 14, 2023 in Magdeburg by Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM) and Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB). In 2015, PPM initiated the first national INSECTA in Magdeburg and since 2016 the INSECTA was jointly organized by PPM and ATB. The annual conference addresses national and international experts from both academia and industry. Our aim is to continue the success of recent congresses and ensure that INSECTA remains the leading insect science and technology conference for attendees and ensures interdisciplinary collaboration.

It has been impressive to see the rapid growth of the insect industry over the past few years. Not only have business sizes increased, but new branches have also joined the existing ones and we are looking forward to welcoming all. New topics have also been introduced to basic and industrial research: allergens and food safety, health benefits, use of artificial intelligence, exploitation of further side streams and many more.

The world, climate and politics are changing fast, as are food habits. New sources for the production of consumer goods are needed in all aspects of life. Developing the insect industry and preparing it for the challenges of the next year will be important parts of our future. For this, we will get together at the conference, where we will learn during the lectures, converse during the breaks, and make new connections at the evening event.

This year’s venue will be the AMO Kultur- und Kongreßhaus. Since the 1950s, the AMO has been one of the most important cultural meeting places in Magdeburg since the Wall between east and west was torn down. A total of around 70 lectures will take place here in three parallel session lines to give all participants the chance to meet their own special topics and discuss with the experts. We are also excited about this year’s exhibition area, where new technologies will be presented.

We would like to express our gratitude to all of our partners, supporters, and sponsors at the ATB and PPM as well as to our sponsors for enabling us to take this year's INSECTA in a new direction.

We would also like to thank the participants who have made INSECTA an important platform for sharing knowledge and discussions of new ideas!

Sara Hadjiali, Thomas Piofczyk, Chandra Dev Borah, Oliver Schlüter

Magdeburg in September 2023

Organizing Team

Dr. Sara Hadjiali
Dr. Thomas Piofczyk
Chandra Dev Borah
Dr. Sarah Bothe
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The ATB is a pioneer and a driver of bioeconomy research. Our vision:
A circular, diverse, innovative and sustainable bioeconomy produces healthy food for all, operates on the basis of renewable raw materials and facilitates the realization of One Health for humans, animals and the environment.

Our research on insects covers the entire supply chain and integrates smart and innovative technologies.
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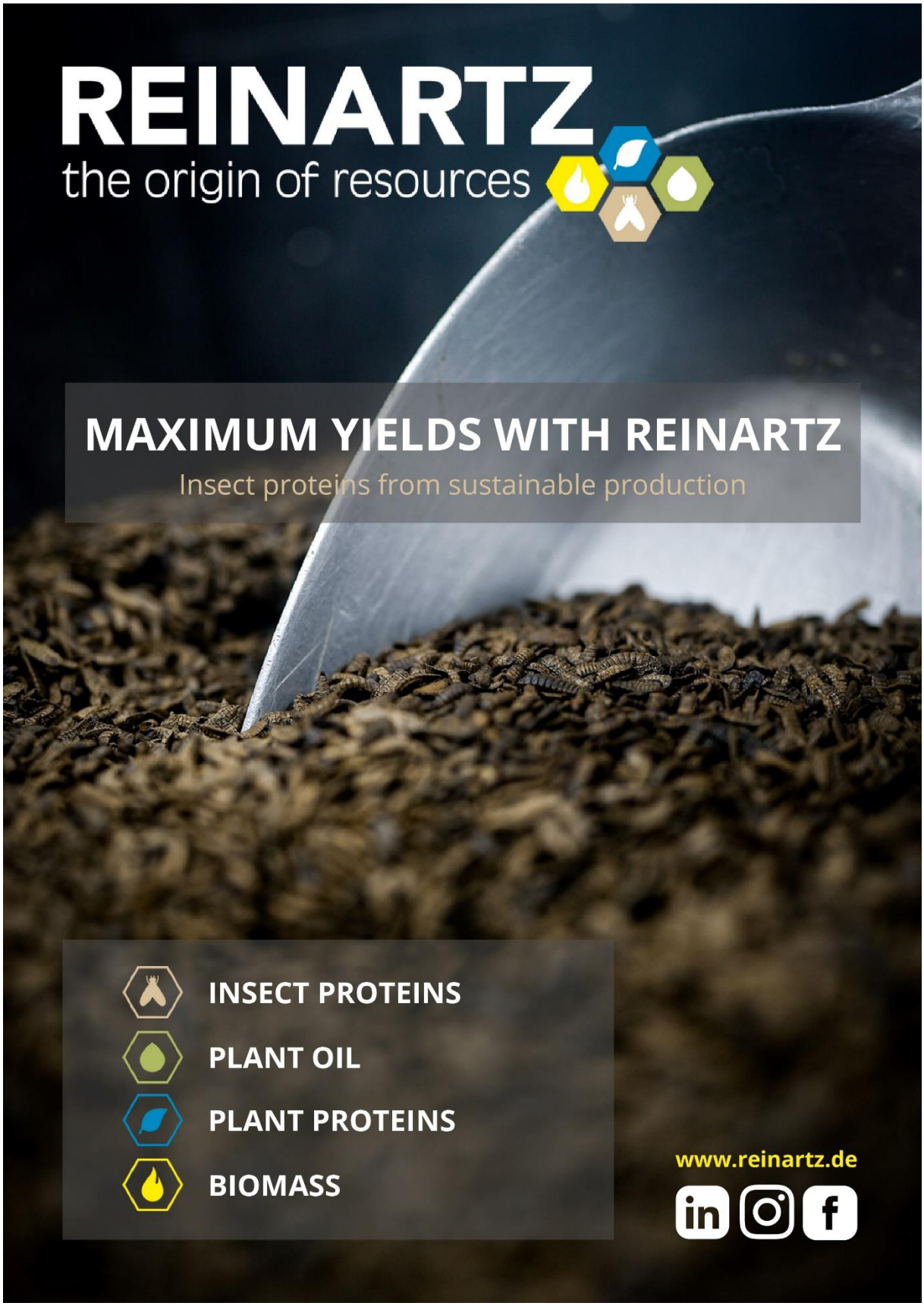
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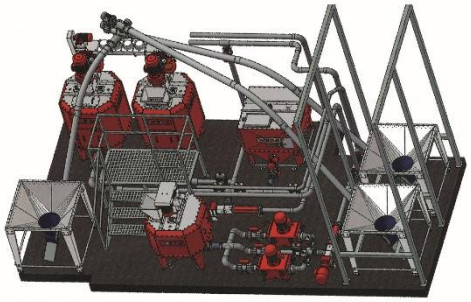

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The VDI is an association for technically and scientifically active people (VDI: Association of German Engineers) and is one of the largest technically oriented clubs and associations in the world. The VDI Magdeburger Bezirksverein supports the INSECTA conference since 2015 and grants the "VDI Best Young Scientists Presentation Award" every year. It is divided into 6 district groups and many thematically different working groups and has offers for all ages. One group related to INSECTA conference is the Arbeitskreis Nachwachsende Rohstoffe (circle of experts for renewable resources) that is managed by Sara Hadjiali. VDI Magdeburger Bezirksverein is organised by voluntary works. The VDI Magdeburger Bezirksverein is supported in its work by numerous companies in the region and forms an important network and the VDI is also international networked. Also, VDI Magdeburger Bezirksverein has a sponsorship award for students at the universities in Magdeburg every year and the Gruson plaque of honor for members who deserved in the technical development and the work of VDI Magdeburger Bezirksverein Magdeburg.

More information can be found at homepage:

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Insect farming in the European Union: intersecting food systems, sustainability, and animal farming

Francis Maugère

Eurogroup for Animals, Brussels, Belgium

As the insect farming sector for both food and feed grows in Europe, the European Union is on the frontline for its regulation. Insect farming for food and feed intersects with the EU Protein Strategy, the Framework for Sustainable Food Systems, and the Farm to Fork Strategy more broadly. The EU is also competent for animal welfare standards.

Yet there are three paradoxes in how the EU is addressing the subject. The first one is that insect proteins are mainly presented as a food solution, while most of the investments in the sector are turned towards insect farming for animal feed. At a time where the Farm to Fork strategy is aiming at shifting European agriculture towards greater sustainability, less intensive animal farming, healthier diets and low-trophic aquaculture, investing in a practice that supports conventional intensive animal farming and greater animal product consumption is surprising. The second one is that insects, although animals, are semantically excluded from animal farming and bundled in with alternative proteins for food consumption, once again ignoring that industrialisation of the insect farming sector is buttressed by animal feed production, and dismissing the welfare issues that arise. The final one is that the EU appears to embrace insect farming as a solution for sustainable food systems, while admitting an overall lack of knowledge surrounding all aspects of the industry, including welfare of the insects, health concerns, capacity to spread pathogens and other risks, and sustainability claims.

Eurogroup for Animals argues that the precautionary principle should be applied to insect farming both in terms of the welfare of the insects in the facilities and in terms of the potential contradiction between increased insect farming for feed and the EU's sustainability objectives for its agri-food system.

The evolution of complete metamorphosis in insects

Christin Manthey

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Metamorphosis, the change (meta) in form (morphe), is a widespread phenomenon in the animal kingdom, where it has evolved independently several times. The most dramatic metamorphic changes occur in the most successful group of animals: the insects, which account for a large proportion of the world's biodiversity. Most insects are holometabolous and undergo complete metamorphosis: they radically remodel their whole body during the pupal stage. The remodelling in the pupa gives the insects the unique opportunity to change their gut microbiota. Comparing larval and adult specimens, holometabolous insects show a higher beta diversity and hence microbiota turnover compared to hemimetabolous insects. These results support the idea that pupation offers the opportunity to change the gut microbiota and thus might facilitate ecological niche shifts. This possible effect of niche shift facilitation could explain a selective advantage of the evolution of complete metamorphosis, which is a defining trait of the most speciose insect taxon, the holometabola.

A second hypothesis for the success of holometabolous insects is that intercalating the pupal stage decouples growth and differentiation. Most growth is confined to the larval stages, while most development occurs in the pupa, allowing for fast larval growth. Comparing the larval growth of holometabolous and hemimetabolous insects, the Holomatabola shows significantly higher growth rates than Hemimatabola. An organism that grows fast pays the cost of compromising the differentiation of the tissues. This could be a driver for the evolution of complete metamorphosis.

Optimizing insect industry with artificial intelligence (AI)

Urs Liebau

Center for digital GreenTech, August-Wilhelm-Scheer Institute, Clausthal, Germany

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The insect industry undoubtedly has enormous sustainability potential. Examples span the way we as humans will feed ourselves in the future, to innovative recycling of waste. The insect industry will thus contribute to closing gaps that still exist in implementing a circular economy. However, in order to take full advantage of this potential, there are a number of challenges that need to be overcome. One key challenge, in addition to the legal hurdles and acceptance of insects as human food, is the optimization of production processes. Digital technologies such as real-time detection, counting and identification of insects can make an enormous contribution here.

My presentation will focus on the opportunities, risks and future applications of AI and other digital technologies in the insect industry. Existing projects and application possibilities will be described and the savings potential through technologies will be estimated. For this purpose, reference will also be made to other industries in which digital technologies are already established in order to draw conclusions on possible application scenarios. The presentation is intended to encourage participants to initiate new project ideas involving digital technologies in production.

Lecture Topics
(All Oral Sessions)

Processing of larvae- Actual trends

Stefan Kirchner, Jörg Heidhues

GEA Westfalia Separator Group GmbH, Oelde, Germany

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Actual most of the farmed larvae will be processed only with drying (whole larvae) or processed into meal and oil.

For the meal and oil processing there are mainly 2 different process types used:

Wet processing and dry processing.

For human consumption sometimes also protein concentrates will be produced, using different processes.

This presentation will compare these process types and highlight the key arguments in respect of product quality, energy consumption and hygienic aspects.

Actual processing trends and variations for the wet process will be discussed.

How to reach your commercial potential and how a supermarket could be unexpected help

Bob Holtermans

Insect Engineers / Insect School, Horst, Netherlands

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The insect industry really started to make its way to becoming a mature industry. One of the biggest struggles is a bit like the chicken and egg story, which came first. The potential customers for insect meal and other related insect products need a relative high volume but that volume is not available. At the same time the insect farmer who is highly motivated can't produce high volumes yet. Because there is that much too learn and getting commitment for sales is then more difficult. Besides that the guarantee of getting enough raw materials year round can also be a topic of uncertainty.

In this playing field a new player comes in, the supermarket. The supermarket can bring an unique perspective to the market. On one hand they are owner of their own waste stream which gives them a guarantee of year round availability of organic waste. On the other hand there is a direct connection with the pet food industry, as pet food is sold in the supermarket.

That means that in practice the organic supermarket waste can be made into feed for the larvae on the black soldier fly farm. The black soldier fly farm sells the processed larvae to the pet food manufacturer. The pet food company then uses the processed BSF larvae as a protein source in their pet food. And last but not least the pet food is sold again in the supermarket. The result a great example of a circular approach.

This approach is not a theoretical approach anymore, currently the biggest UK supermarket is involved with building the first commercial BSF farm. Which is key to help to industry forward. Because the industry needs a minimum production volume if it wants to be taken serious by large potential players. Perhaps the chicken came first, perhaps the egg came first, but in both cases the supermarket can be the missing piece of the puzzle.

Dielectric drying of black soldier fly larvae: impact on nutritional, chemical and microbiological quality and stability

Dries Vandeweyer, Dario Lachi, Mik Van Der Borght

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Drying is one of the most important stabilisation methods for insects for food and feed. Typical drying methods such as hot air drying or freeze drying have already been successfully applied in the insect sector. However, these processes have high energy requirements, can be time-consuming, and, especially in the case of hot air (oven) drying, may reduce product quality. More innovative drying technologies, such as dielectric drying, may prove to be useful as a lower-cost and quick alternative that minimally affects product quality.

Within the SUSINCHAIN project, two dielectric drying technologies, namely microwave and radio frequency (RF) drying, were validated for drying black soldier fly (BSF) larvae (*Hermetia illucens*) on an industrial scale. After optimising the equipment and process parameters, a large-scale experiment was conducted: a large batch of (blanched) BSF larvae was dried using both dielectric drying methods, while chemical, nutritional, and microbiological analyses were performed to assess quality and stability of the larvae. As benchmark technologies, oven drying and freeze drying were applied to the same batch of insects. Thus, the impact of both dielectric drying technologies on macronutrient, amino acid, fatty acid, and mineral composition, vitamin B12 content, lipid oxidation, microbial counts, and the presence of pathogens was monitored and compared to the impact of freeze and oven drying. Additionally, a shelf life test comparing both dielectric drying methods was conducted to track chemical and microbiological stability.

Results showed that, in general, differences in nutritional parameters were minimal when comparing dried BSF larvae from the four drying technologies. However, lipid oxidation and microbiological quality of the dried larvae varied significantly among the four technologies. Interestingly, dielectric drying could further reduce total viable counts and bacterial endospores compared to oven- and freeze-dried insects. In conclusion, dielectric drying of BSF larvae can produce high-quality dried insects and, depending on the application, both RF and microwave drying have their specific advantages.

CO_2 and ammonia emitted by *Tenebrio molitor* and *Acheta domesticus*

Coudron C. L.[1], Berrens S.[2], Van Peer, M.[2], Deruytter, D.[1]

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[2]*Thomas More, Kleinhoefstraat 4, 2440 Geel, Belgium*

Insect production systems are often proposed as more sustainable compared to conventional livestock farming. However, sustainability can be a vague umbrella term. This study covers one part, carbon dioxide emissions during insect rearing. Besides the impact on our climate excessive CO2 concentrations have detrimental effects on the health and safety of the farmers. HVAC systems can provide fresh air, and avoid high CO2 concentrations but they can be expensive and should be dimensioned adequately. Yet, little information is currently available.

In order to partially overcome this knowledge gap, research was performed to determine CO2 emitted by Tenebrio molitor and Acheta domesticus throughout their production processes. CO2 emissions were measured using an accumulation chamber. A batch of insects is placed inside the chambre and gasses are allowed to accumulate. The accumulation chambre collects a data point every pre-set time interval. From this timeseries data one coefficient is estimated. This is an estimate of the amount of CO2 that is produced by one kg of live insects per hour. By repeating this several times throughout insect growth, the age dependent biomass specific CO2 production can be modelled.

Up to 40 mg mealworms and house crickets have very similar CO2 emission rates. From then on the rate at which CO2 is emitted increases almost linearly with an increasing average weight of the crickets but slows down for mealworms. The models estimate the total amount of respiratory CO2 to produce one kg of live insects on control feed between 769 and 854 g (95% CI) for mealworms and between 388 and 530 g for house crickets.

Cultural influences on insect food acceptance: A comparative study in Europe

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The integration of insects into conventional diets offers significant potential for sustainable food production and tackling global food security challenges. However, despite their nutritional value and environmental advantages, incorporating insects as a regular part of diets encounters substantial obstacles. Cultural factors have been identified as influential elements that impact the acceptance of unconventional food sources, including insects. This research focuses on understanding the acceptability of insect foods in five European countries: Germany, the Netherlands, the United Kingdom, France, and Romania. These countries represent a wide range of cultural backgrounds within Europe. To provide familiar and accessible options, burger patties and other commonly consumed products were selected as the primary food format for evaluation. The research was conducted through online surveys distributed via social media channels. By examining consumer preferences and cultural influences, our objective is to identify key factors that can contribute to the successful integration of insect foods into European diets. Our findings highlight the diverse preferences and cultural nuances observed among the studied nations. These differences necessitate the development of tailored strategies to overcome cultural barriers, which are crucial for the successful implementation of acceptance strategies. The significance of our results lies in assisting the industry in formulating effective approaches to enhance consumer acceptance of insect-based foods, thereby promoting sustainable and alternative food sources in Europe.

Impact of enzymatically treated substrate on insect development and survival

Michał Krzyżaniak[1], Olga Kosewska[1], Łukasz Grabek[1], Kazimierz Warmiński[1], Mariusz J. Stolarski[1], Daniel Maurer[2], Manel Ben Amor[2], Andreas Wilke[2], Thomas Eisele[2]

[1]*University of Warmia and Mazury in Olsztyn, Olsztyn, Poland*
[2]*Offenburg University of Applied Sciences, Offenburg, Germany*

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The world population is predicted to increase for the next three decades and will reach more than 10 billion people. Therefore, food sources other than traditional food crops and animal livestock are being sought as a solution. Insects could be an affordable and nutritious alternative to traditional resources. It is proposed to use the commonly available lignocellulosic biomass as a potentially new source of feed for insects. Therefore, the aim of this research is to determine the effect of pretreated lignocellulosic biomass (wheat straw and cup plant), which is usually not applicable for insect farming, on development and composition of yellow mealworm (*Tenebrio molitor* L.). Four different lignocellulosic substrates where used (pretreated with steam explosion, the organosolv process, enzymatic hydrolysis and their combination) as a sole insect feed or mixed with wheat bran (also used as a control). The preliminary results show that individual larval weight at 11 week of larvae growth was the highest for larvae reared on wheat bran only (132 mg) and the lowest for that reared on wheat treated with organosolv (1.33 mg). In general, the more lignocellulosic materials the less was the insect weight. Survival of reared larvae on processed lignocellulosic substrates was very low (0-67%), and the addition of wheat bran significantly increased it. The research will be continued to determine the best feed mix and the physicochemical characteristics of mealworms as feed or food.

Acknowledgement

These results are a part of research project entitled “Growth performance, chemical composition and valorisation of residues of yellow mealworm fed with pretreated lignocellulosic biomasses“ (2GenBug) funded by National Science Centre, Poland, grant no. UMO-2020/39/I/NZ9/00907.

Enhancing nutrient-poor substrates through Solid-State Fermentation: The impact on nutritional value and the fate of secondary metabolites

Wael Yakti, Marcus Müller, Nadja Förster, Inga Mewis, Christian Ulrichs

Humboldt-Universität zu Berlin, Berlin, Germany

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Insects-based bioprocesses can generate value by transforming low-quality by-products into high-quality insect biomass. Improving the performance of insects on nutrient-poor substrates, or improving the nutritional value of the substrates for the insects, is particularly important given the nutritional demand of the insects. In our facility, black soldier fly larvae (BSFL) and mealworms (MW) are grown in a circular production system that connects modules of plant production, aquaculture, and insect rearing, forming a circular production system (the CUBES). The production modules of this circular system accumulate significant amounts of low-value side streams, such as plant leaves, roots, and used hydroponic substrates (from the plant module), and fish feces (from the fish module). These side streams are rich in fibers with low macronutrients content making them difficult to utilize as substrates for insect rearing. Additionally, they contain antinutrients (in the case of plant leaves) and substantial amounts of heavy metals (in the case of fish feces). To address these issues, fungi-based solid-state fermentation (SSF) processes were developed for these different side streams. This presentation focuses on the potential of using fungi SSF to break down antinutrients and fibers, and enhancing the macronutrient content in the substrates. The SSF processes are followed by BSFL and MW feeding trials to assess the benefits of SSF bio-treatment in promoting larval growth and the accumulation of specific health-relevant secondary metabolites.

Comparative assessment of insect processing technologies for sustainable insect protein production

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Considering the projected increase in demand for protein sources, finding alternative sources with lower environmental impact has become of great importance. Insect mass production has emerged as a potential solution, particularly in Western countries. However, the insect industry requires environmentally optimized and economically efficient value chains. Despite the increasing number of studies on insect production, data availability remains a challenge, with aggregated data that lacks transparency in comparison to well established feed and food industries. Previous environmental studies indicate that insect farming and processing stages are responsible for most of the environmental impacts associated to the final product. This study aims to compare the environmental impacts of traditional and alternative processing technologies for insect protein production and determine the most environmentally friendly process. Life cycle assessment (LCA) was used to evaluate the environmental impacts associated with insect farming and processing following the Environmental Footprint (EF) methodology. Most relevant impact categories for insect production and processing were identified as land use, energy use, freshwater ecotoxicity, water use and climate change. Among the processing treatments evaluated, FOP (freezing-oven drying-hot press) showed the best environmental performance in all selected impact categories while the BOS (blanching-oven drying-SFE with CO_2) group had the highest environmental impacts in all categories. Results from this study indicate that the environmental impacts of insect processing can be reduced by using alternative technologies. These findings underscore the importance of carefully selecting processing technologies to reduce the environmental impacts of insect protein production.

Preparing students of veterinary medicine for attending insect farms in Germany

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Insect farming is not an option, it is reality. With productive insects granted the status of livestock in the EU, it is necessary to provide veterinary services. While public health veterinarians have been made aware of them over the last years, practitioners frequently do not know how to attend an insect farm. In 2019, a first online seminar was offered during a congress in Germany. Single lectures about food insects have been held for many years as part of the general curriculum, but instructions regarding herd management for students is a novelty in Germany.

During a project founded by the program “Freiraum” of the StIL, students of all semesters could enrol in a one-semester elective course in insect herd management. It was limited to 12 students and was quickly oversubscribed. The course consisted of 2 hours/week of theoretical and practical instructions. In addition, groups of 2-3 students were tasked to take care of batches of Mediterranean crickets (*Gryllus bimaculatus*), from hatching to harvest. Each group had to feed its batch regularly with a self-chosen diet; they recorded the batch’s progress by weight and instar classification. Animals were harvested and processed as food that was offered at the final session, where each group reported on the development of their batch.

Instructions dealt with the basics, anatomy, physiology, pathology, immunology, and infectious diseases; followed by specific herd management advice for xiroculture, hygroculture, aquaculture, and xyloculture. Theoretical lectures and discussions were complemented by practical activities incl. sexing darkling beetles, swabbing and cultivating of the microbiome, necropsies, and sample taking.

Academic staff were both biologists and veterinarians, aided by student assistants. To showcase the global perspective, online sessions with (inter)national experts in the field of productive insects were arranged in English and in German.

Feedback from the students, the print media and television was very positive. All the students would recommend the course to their friends. Most of them claimed that the caring for their own crickets was the best part of the course. However, none of them rated the relevance for their future career as high or even medium.

Selective breeding in black soldier fly and what this can achieve in productivity

Desmond Cave

Beta Bugs, Easter Bush Campus, Roslin, United Kingdom

Protein for human consumption is unsustainable using the current methods of farming animals such as chickens, pigs, sheep or cattle. The production of Black Soldier Fly (BSF) or the meal produced from the drying and milling of the larvae has become an important factor in the production of protein worldwide. Farming BSF larvae is sustainable, produces less CO_2 emissions, uses less water and can use feedstocks which would not or cannot be used by other farming techniques.

At Beta Bugs we have been selectively breeding the Black Soldier Fly (BSF) for over 4 years. The traits we have selected for are: Larvae mass at 12 days of age, development time from hatch to harvest and the number of eggs laid per female fly. We will describe how the traits were selected, the importance given to each, how they interact between each other and the basics of the selection procedure.

We now have a High-Performance Larvae – HiPerFly. We will describe the changes we have been able to make in the HiPerFly when compared to the standard fly and will compare larvae mass, protein content, development time and egg production. We will also describe what benefits this provides to a farmer growing BSF in terms of quantities of larvae, protein and economic profit. We will also try and evaluate what this means for the global production of BSF.

Scaling up fly mating chambers: lessons learned from operating 4m^3 and 24m^3 fly mating chambers

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InsectoCycle has been dedicated to developing large-scale black soldier fly reproduction units. These units feature a fly mating chamber (light cages) that is either 4m^3 or 24m^3 and have been in operation for the past two years. Compared to typical laboratory scale experiments, these units are notably larger, resulting in new interactions and diverse microclimates within the cage. In-depth research has been conducted to comprehend the key factors that impact fly mating and egg deposition, as well as how to measure and influence these factors. Some of the factors that have been examined include air temperature, surface temperature differences between the floor and walls, humidity at the floor versus the ceiling, airflow through the cage, and the necessary refreshment rate to regulate CO2 and ammonia levels for optimal performance. However, controlling these elements simultaneously can be challenging and determines the success and stability of the reproduction process.

Insect fat of *Hermetia illucens* as base material for the production of biolubricants

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Black soldierflies are used to convert organic raw materials into valuable insect biomass on an industrial scale. The resulting insect meal consists of insect fat and protein. Insect proteins are mainly used as animal and pet food. It has been shown that insect protein meal can partially replace conventional feeds such as fishmeal or soy in animal feeds and can have a significantly better environmental performance than conventional protein feeds.

Insect fats are a by-product of the Hermetia protein production process. The production of biolubricants from Hermetia fat appears to be feasible based on the properties of the fat. Applications for biolubricants can be found in almost all technical fields, especially in environmentally sensitive areas. These include forestry, construction, shipping and waste water management.

Preliminary laboratory studies indicate that the ratio of unsaturated to saturated fatty acids in insect fat and the acid spectrum can be shifted towards specific high-value monounsaturated fatty acids through insect feed selection. This holds the potential for standardized production of raw insect fat with an optimized fatty acid composition as a raw material for biolubricant production. In order to study the relationship between feed material and insect fat composition, experiments were carried out with saturated and unsaturated fat additives.

The presentation will show the results of material characterisation tests on Hermetia fat and the specific properties of the raw material. Experimental results on biological control of the fatty acid composition of Hermetia insect fat will be presented and a refining concept for cost-effective production of novel bio-based and biodegradable lubricants discussed.

Degradation of bioplastics by black soldier fly (*Hermetia illucens*)

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Bioconversion by *Hermetia illucens* (Diptera, Stratiomyidae) larvae is a novel technology for manures (poultry, swine, cattle) treatment and valorization in agriculture. However, some microparticles from bioplastics present in manures may have negative effects on larval production. Moreover, its efficiency of manures degradation may also be affected by microplastics (MPs).

In this study, three types of bioplastics were tested in the experiment: (i) polylactic acid (PLA) (ii) Polybutylene succinate (PBS) (iii) Polybutylene adipate terephthalate (PBAT). Theses bioplastics were grinded into small particles. About 1% of MPs was mixed into substrate rearing (poultry and swine manure). These substrates spiked by MPs were digested by chemical extraction and analyzed by FTIR, TGA and RMN.

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 contains 170 larvae 3days old and 300 g of substrates (1/3 poultry manure and 2/3 swine manure). The larval density is one larva by 1 cm^2 (based on literature data and our preliminary trials). The substrates were not enriched with additional substances such as water or feed.

In each boxes, we have respectively detected by FTIR in frass analysis, 78% for PLA, 74,8 for PBS and 78,2 % for PBAT. No trace of bioplastics were found in control box.

For PBS and PBAT, no changes on bioplastics structure were detected by different analyses (FTIR, TGA and RMN). However, some variations in molar mass of PLA were detected by TGA analysis. This variation could be due by insect activities.

Effects of *Hermetia illucens* frass on growth and development of bean plants *(Phaseolus vulgaris* L.)

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Industrial scale insect production generates large quantities of residue or frass, a mix of unconsumed substrate, insect parts and excrements. To enhance the sustainability of insect rearing, the frass should be valorised. Evidence exists it could be used as soil improver, fertiliser and/or health promotor in plant cultivation. EU legislation imposes heat treatment prior to use to ensure consumer safety but this process might compromise the chemical and microbiological properties underlying the frass plant-growth promoting potential.

The effect of frass from *Hermetia illucens* larvae rearing on development of bean plants (*Phaseolus vulgaris* L.) was studied by means of vegetative and generative growth parameters. Test plants grown 9 weeks under controlled conditions in frass-potting soil mixes (0.5, 1, 2 and 4% fresh or dried (1h 70°C) frass) were compared to control plants grown in potting soil. The effect of drought stress (50% water supply reduction) was studied in a similar set-up for the 2% mix.

Under normal conditions frass addition did not seem to alter bean plant vegetative growth parameters such as leave number, plant height and fresh/dry weight. However, the pod dry weight seemed to increase, with a significant result for addition of 4% dried frass. In accordance with these observations, photosystem related parameters such as F_v/F_m and PI_{abs} and specific energy fluxes in the transport electron chain indicated improved light capture and processing in bean plants which received either 2 or 4% fresh or dried frass.

Under drought stress, addition of frass seemed even more important. Bean plants not only showed improved light reaction efficiency, but also higher leave number, plant fresh/dry weight and pod dry weight compared to control plants.

In conclusion results indicate improved performance of bean plants grown in 2 or 4% *Hermetia illucens* frass/potting soil mix under normal conditions. Also under drought stress, frass addition seemed to improve plant growth and pod production. Heat treatment of the frass did not seem to influence its effects on plant growth.

Practical adjustments in fly larvae composting when treating plant-based waste

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Plant-based wastes (pbw) are often challenging materials to treat biologically, due to their high fiber content. Black soldier fly larvae (BSFL) might be an efficient technology for bioconverting these wastes into added-value products. This study investigated the bioconversion of greenhouse waste (cucumber plants after harvesting with TS=13%) with BSFL. Four treatments were investigated: pbw with enzymes (E), pbw with frass (treatment residue from BSFL composting of pig feed) (F), pbw with enzymes and frass (EF), and a control with only pbw (C). In addition, two extra treatments with half of the amount of feed and larval density were established using the EF as base (H) and the other using EF substrate with addition of a foam plastic lid covering the pbw with small gaps at the edges (HT). The feed dose was 0.2 g of volatile solids (VS) per larva in every treatment. Bioconversion efficiency (BCE), material reduction (Mat.Red), larval survival and possibility to dry harvest larvae at the end of the treatment were evaluated. All treatments had a survival rate of >92%, except treatment C that had a survival rate of 77±3%. Treatment C had the lowest BCE_{VS} (6±0.3%), $Mat.Red_{VS}$ (34±5%) and the harvesting was difficult due to wet residue with high content of unprocessed material. Adding frass (F) or enzymes (E) to pbw increased both BCE_{VS} to 8±0.5% and 8±0.7%, respectively and $Mat.Red_{VS}$ to 45±1% and 46±4%, respectively. Adding both (frass and enzymes) did not further increase BCE_{VS} (8±0.3%) nor $Mat.Red_{VS}$ (46±1%). Neither of these amendments in the treatment resulted in a simplified harvesting. When the amount of feed was reduced by half, no differences were found in $Mat.Red_{VS}$; however, BCE_{VS} increased to 10±0.7%. The most significant changes occurred when a foam mat was used to cover the boxes. With the lid (treatment HT), a BCE_{VS} of 14±0.7% and $Mat.Red_{VS}$ of 54±2% was achieved. In addition, larval harvesting was simplified. However, the amount of waste treated per cm^2 in HT was lower, so there is a trade-off between treatment efficiency and treatment capacity (1580 kg per treatment in a container). In conclusion, pbw is a substrate that requires amendments if it is to be used as feed substrate as it is not an optimal substrate in BSFL rearing, as the treatment capacity achieved was low.

BSF health and welfare: Emerging challenges for the industry

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Among the challenges faced by the insect-rearing industry, two have received limited attention: health and welfare. Indeed, BSF has traditionally been considered highly resistant to infectious disease and until recently, there were no published reports of naturally-occurring outbreaks. As for welfare, insects have been typically classified as non-sentient, thus lacking consciously-experienced states such as pain, and given this, falling outside the sphere of moral concern. The insects for food and feed industry currently operates with relative freedom for these topics; having for example no reportable diseases, nor insect welfare legislation.

The aim of this talk is to present these emerging challenges in the context of mass-produced BSF and highlight the remaining scientific gaps. It is based on the review of about 140 scientific publications published until Spring 2023.

The recent literature demonstrates that BSF, like all other livestock, are susceptible to health risks posed by pathogens and exogenous pests. These risks are potentiated by a multitude of factors, and currently, diagnostic and treatment options are immature. Indeed, recently described pathogen and pest outbreaks in BSF caused major losses while novel approaches indicate the existence of BSF-specific viruses.

There is increasing evidence for insect sentience and welfare concerns are present across all stages of production and processing. For example, larvae may experience thermal discomfort in densely-populated bins, while adults still living at the end of a production cycle must be euthanized; welfare-compatible, efficient euthanasia and slaughter techniques for insects in mass-production systems should be part of the discourse. In some countries, regulations have recently evolved to include previously-excluded invertebrates based on literature showing their sentience; this trend may eventually include insects, too.

Both literature reviews reveal that commonly-held assumptions pertaining to BSF health and welfare must be carefully reconsidered and the creation of a sustainable BSF-rearing industry requires proactive actions. They are synergistic; in improving one, parallel gains can be expected in the other. Promoting these topics on an industry-wide basis by forming consortiums, preparing guidelines, auditing tools, and collaborating on research efforts can be a win-win for all involved.

Microbiological profile of productive gryllid and tenebrionid frass before and after heat treatment

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From the regulatory point of view (REG [EU] 142/2011) and unlike other domestic animal manure, insect frass must be submitted to heat treatment (min. 70 °C for one hour) before usage. The objective of this study was to evaluate this treatment in terms of microbiological safety.

A total of n = 22 samples from productive gryllids (*Gryllus assimilis* and *G. bimaculatus*) and tenebrionids (*Tenebrio molitor* and *Zophobas atratus*) were analysed microbiologically (*Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, total aerobic mesophilic bacterial count [TBC], Enterobacteriaceae, *Bacillus cereus*, coagulase-negative staphylococci [CNS], moulds, and yeasts; standard DIN-ISO methods) as taken from harvested lots ("untreated") and after being heated in a water bath at 70 °C for 60 minutes ("treated"). Animals had been fed with season's vegetable off-cuts and chicken feed (gryllids) and wheat bran (tenebrionids). Data was transformed into decadic logarithms and analysed with Student t Tests.

No *Salmonella* spp., *L. monocytogenes*, *E. coli*, nor *S. aureus* were found. Highest counts were TBC (8.309 ± 0.840 $\log10_{cfu/ml}$). Species-related, significant differences among untreated samples occurred in all parameters but TBC and CNS, e.g., gryllid frass yielded more Enterobacteriaceae and *B. cereus* than that of tenebrionids. Thus, and as expected, each species' frass had a specific microbiological profile.

Heating significantly affected microbial growth. The degree of germ reduction varied markedly, from 0.164 ± 0.235 (*B. cereus*) to 5.106 ± 1.574 $\log10_{cfu/ml}$ (yeasts). While the actual levels of the bacterial counts in untreated and treated frass varied with the insect species and the microorganism, the degree of reduction only depended on the microorganism. Fungi and Enterobacteriaceae were effectively reduced or even vanquished. Reducing CNS was also relatively efficient (2 – 3 log10 stages), but TBC was rather stable, and *B. cereus* was practically not affected by this method.

Insect microbiology is species-specific, also in frass. The species determines the initial microbiological profile and counts, while heat treatment reduces them in constant ways, leading to reduced (but still species-specific) counts after heat treatment.

Transmission of microorganisms across different life stages of black soldier fly (*Hermetia illucens*): Case study of *E. coli*

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Since black soldier fly (BSF) larvae can be reared on organic waste streams with high microbial load, microbiological safety should be monitored when producing these insects for food and feed purposes. Besides horizontal transmission of food pathogens (from substrate to the larvae), vertical transmission of food pathogens across different life stages of BSF is important to understand.

In this study, 8-day old larvae were reared on chicken feed inoculated with about 7.0 log cfu/g of *E. coli*. A second inoculation with *E. coli* was conducted at day 18. Uninoculated chicken feed was used as control. Substrate and insect samples were taken every three or four days until the end of the adult (fly) stage. Besides counts of *E. coli*, general microbial counts (total viable count, Enterobacteriaceae, lactic acid bacteria, aerobic endospores, and yeast and molds) were determined.

The control cycle showed retention of most microbial counts until the transition from pupa to adult, where a decrease of all counts was observed. When reared on a substrate contaminated with *E. coli*, the larvae appeared to ingest the pathogen in high amounts, but reduced the counts to approximately 2.0 log cfu/g in their body after ten days. After a second inoculation of the substrate of the 18-day-old larvae, the larvae even reduced *E. coli* counts to 1.8 log cfu/g after four days. While *E. coli* was transmitted in low amounts (< 2.0 log cfu/g) from larvae to prepupae, further reduction of *E. coli* counts was observed during the (pre)pupal stage. *E. coli* counts were even below the detection limit (1.0 log cfu/g) in the adult stage. Furthermore, the larvae were able to reduce *E. coli* counts in the substrate to < 4.5 log cfu/g after both inoculations.

Although it can be concluded that *E. coli* was transmitted until the (pre)pupae stage, vertical transmission of ingested *E. coli* was not observed until the end of the rearing cycle. Yet, it can not be generalized that vertical transmission of pathogens in BSF does not pose a problem regarding food and feed safety. Several factors, such as pathogen species, insect species and substrate, can influence possible transmission across life stages, demonstrating the importance of case studies.

T. molitor in circular economies: Key aspects of the implementation of *T. molitor* mass rearing in Austria

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Implementing insect mass rearing in an agriculture which is consisting mainly of small-scale farms, such as it is the case in Austria, is a challenging endeavor. Even though *T. molitor* mass rearing has the advantage of space requirements and frugality, legal frameworks, price policy and the availability of suitable feeds still represent considerable challenges. In this study, the latter issue is addressed, quantifying available and suitable agricultural by-products of the Austrian agriculture and food industry, which can be utilised for fattening *T. molitor*. The results of feeding trials are presented and perspectives of *T. molitor*, integrated in an Austrian circular economy are given.

Innovative entomological solutions for dietary supplements

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The BEWARE INSECTA project focuses on studying *Tenebrio molitor* as a novel protein source. Its objective is to optimize the insect flour production process, investigating various farming parameters' influence on nutritional composition. The aim is to develop a protein-rich dietary supplement that meets the specific needs of the elderly and athletes in terms of proteins and amino acids.

In the study, mealworms were reared in specially designed 40x60cm breeding tanks with controlled climatic conditions (27°C, 60% RH). Locally produced organic wheat bran served as basic substrate, with agar (25g/L) as water source. Four test substrates and a control substrate were evaluated from the 2nd month of larval development. The formulations used were as follows: i) control : 100% wheat bran, ii) ratio A: wheat bran and pea protein hydrolysate (9:1), iii) ratio B: wheat bran and pea protein hydrolysate (8:2), iv) ratio C: wheat bran and pea protein hydrolysate (2nd month 24:1; 3rd month 7:3), v) ratio D: wheat bran with pea protein hydrolysate and buttermilk powder (2nd month 21:4:0; 3rd month 10:3:7). All recipes were tested in triplicates.

Eggs were laid in organic rebullet, and the larvae were transferred to the breeding tanks at 4-w.o. at a density of 6.7 larvae/cm² using the test substrates. Mortality tests were conducted on mature larvae and nymphs during each cycle, with 100 individuals used for each test. At the beginning of each nymphal stage (between 80 and 110 days), larvae were harvested, and 50 individuals were weighed to calculate average weight and survival rate.

Individual weight variations were observed among different groups due to varying nutritional inputs, particularly protein and carbohydrate levels impacting mass gain. A variability in the results was observed, that needs to be further investigation. Future steps include the dosage of proteins and amino acids of larvae fed with various substrates, as well as microbiological and toxicological tests. INSECTA aims to develop specific and stable nutritional quality specifications for insect flour production, along with adapted protocols for nutritional analyses in the insect matrix.

Modified wheat-bran-based artificial diet for mass culturing of mealworm (*Tenebrio* sp.): Implications on its biomass quality

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The utilization of yellow mealworm for food and animal feed is gaining interest globally. This study assessed the growth performance, survival rate, bioconversion rate and nutritional composition of mealworm larvae fed on wheat bran (WB) with different inclusion levels (25, 50, 75 and 100%) of potato waste (PW).

The results indicated that mealworm larvae fed on diets with 25, 50,75% PW had increased body length and higher final weight gain than those fed on sole diets (WB, PW alone). The survival rate of mealworm on the various diets ranged between 92 - 94%. Mealworms fed on diet with 75%PW had feed conversion ratio of 1.9. The efficiency of conversion of ingested feed values increased with increasing inclusion levels of PW. The crude protein and fat of mealworm larvae fed on the various waste recipes ranged between 43 – 55% and 35 – 47%, respectively. The energy content of the processed mealworm meal was higher (609 Kcal), when the larvae were fed on diet with 75%PW. No significant differences in crude fiber and neutral detergent fiber were observed for larvae fed on the various diets. The acid detergent fiber of mealworm meal was 2-3 folds higher for larvae fed diet with 100% WB. Considerable amounts of lysine (1.6 – 2 mg/100g), methionine (0.5 – 0.7 mg/100g), leucine (1.4 – 2 mg/100g) and threonine (0.8 – 1 mg/100g) amino acids were detected in the mealworm larvae.

Our findings revealed that using cheap agricultural byproducts (WB and PW) as the basic material for an artificial diet for mealworm to ensure optimal growth and development is feasible, thus substantially contributing to reduced rearing costs and promoting the development of new and promising recipes for their mass production in Africa and beyond.

Nutritional, sensory and microbial characteristics of extruded millet and maize composite flours enriched with house crickets (*Acheta domesticus*)

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Low nutrient dense thin cereal-based porridges (10% w/v) are commonly eaten as beverages or weaning foods. Edible insects such as house crickets (*Acheta domesticus*) could improve porridge flour nutrient density. However, the form of inclusion in porridge flours and processing conditions have been hardly studied. Cricket-enriched composite cereal flours extruded at different temperatures (120, 140 and 160 °C) were characterized.

Two formulations were made with house crickets, whole grain maize and millet flours in the ratios of 66.7:25:8.3 and 66.7:29.2:4.2 for the undried and dried cricket containing formulations, respectively. Each of the formulations were divided into three portions and extruded using a twin-screw extruder to yield six products which were characterized for nutritional (Proximate, iron and vitamin B_{12}) and microbial parameters (spoilage and pathogenic microorganisms). A control product without crickets was formulated and used for comparison. Sensory acceptability and preference tests were only done for the latter and the control porridges without crickets. Mean differences were determined by ANOVA and post-hoc tests.

Generally, key nutrients ranged from 11.69 – 13.28, 0.025 – 0.031 g/100 g protein and iron, respectively and 1.20 - 1.63 µg/100 g vitamin B_{12}, with protein content significantly (p = 0.00) higher in dry-cricket containing flours. Generally all microbial counts were within safe levels.The undried formulation extruded at 140 °C had more protein and vitamin B_{12} than the control; lower microbial counts except significantly higher amounts of aerobic spore formers and was more preferred than the control ($p < 0.05$). Results can be attributed to formulation differences and processing conditions.

In conclusion, extruded cricket enriched flours contained more nutrients and were moreacceptable than the control.

Implementation of chitin analysis in the Weende analysis: A cost-effective approach for assessing chitin content in insect-based feed ingredients and mixed feed

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Insects are increasingly being used as a sustainable and protein-rich ingredient in the diets of fish, pets, pigs, and poultry. Insect meals offer a favourable nutritional composition, containing essential amino acids, minerals, and fatty acids that are beneficial for animal nutrition. However, one significant difference between insect meals and conventional protein sources is the presence of chitin. Chitin has an impact on the determination of crude protein in these products. The nitrogen content within chitin, accounting for approximately 6.89 % of its mass, is detected as crude protein during analysis, leading to an over-estimation of the actual crude protein content. In the traditional nutritional analysis method (Weende analysis), chitin is classified within the fibre fraction, along with other polymeric compounds.

The objective of this study is to analyse the chitin content using a method that is cost-effective and does not require specialized equipment like LC/GC-MS, making it suitable for insect production and processing industries. The proposed analysis technique is based on classical chemical methods such as crude fibre and nitrogen content, allowing for easy implementation within existing feed analysis practices. The process involves treating the sample with a 0.25 M sodium hydroxide solution, followed by drying the residue at 103 °C for 4 h. The nitrogen content of the residue is then determined, and the chitin content is calculated stoichiometrically based on the nitrogen measurement.

During the method validation, a recovery rate exceeding 95 % and a standard deviation below 5 % were achieved, indicating the reliability and precision of the method. The method demonstrates the capability to detect chitin content within relevant levels (up to 2 %) in feed samples with a measurement uncertainty below 10 %. The method was successfully applied to determine the chitin content in various products derived from insect breeding and processing, including three insect species and different developmental stages of the yellow mealworm (*Tenebrio molitor*), such as larvae, pupae, and beetles. Furthermore, feed products like wet dog food and dry cat food were also analysed. These findings enable an estimation of the insect protein content, provided that the raw material is known.

Biotransfer of heavy metals along the edible insect-human food chain: Implications for consumer safety

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Edible insects have emerged as a promising alternative food because of their high nutritional value and low-ecological footprint. However, the safety of wildly harvested edible insects is threatened by environmental pollution, particularly with heavy metal from mining companies. This study investigated the concentrations of eight heavy metals [arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn)] in wild harvested edible insects in the Copperbelt province, Zambia.

The potential health risks involved in their consumption are discussed. Soil, plant and edible insect samples were collected along a 60km pollution gradient. Atomic Absorption Spectrophotometry was used to determine heavy metals concentrations.

Our results revealed higher concentrations of heavy metals accumulation in the various edible insect species: *Cirina forda* [Ni (70 – 81%)], *Imbrasia obscura* [Cd (2 – 84%); Pb (10 – 79%)], *Cirina forda* [Fe (63 – 96%)] *and Macrotermes falciger* [Zn (5 – 57%)], when compared to other species. The quantity of *Imbrasia obscura* consumed (248 g person^{-1} day^{-1}) was significantly higher (9 – 37%) than other insect species. The consumption of insects was directly associated with increased daily intake of heavy metals, which enhanced the target hazard quotient, and increased the associated health risks up to 9 folds when compared to World Health Organization (WHO) permissible limits.

Therefore, the potential of heavy metal mobility in the consumer trophic levels and the ecotoxicological consequences are particularly concerning given that over 92% of edible insects consumed in Zambia are harvested from the wild. This risk associated with edible insect value chain can be significantly reduced by promoting captive mass production of insect for food to ensure safety, quality and availability for improved food and nutrition security.

Dietary protein levels affect health, development and immune responses of black soldier fly

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Dietary composition influences the growth and development of black soldier fly (BSF). The level of macronutrients, specifically proteins (P) and carbohydrates (C), in insect diet are critical for weight gain and body composition (proteins and lipids) of the larvae and also for development to the adult stage. The BSF larvae are capable of growing on a wide range of dietary C levels, however, dietary P levels can be limiting for growth and development.

The effect of dietary P level on larval survival and health during bacterial infection and development to the adult stage in BSF has not yet been investigated. Larvae of BSF were reared on three experimental diets whose dietary C levels were fixed at 50%, while their dietary P level varied (P:C ratios: 1:5 (designated as low); 1:2.2 (medium); 1:1.42 (high)). To determine the effect of dietary P level on insect immunity, survival of five-day-old BSF larvae was recorded after injection with the Gram-negative bacterium, *Pseudomonas protegens* strain Pf-5. The effect of dietary P level on larval health was assessed by quantifying the level of uric acid accumulation in larval hindguts. The effect of dietary P levels on development to the adult stage was evaluated by determining the duration until adult emergence and percentage reaching adulthood.

Our findings show that high dietary P level resulted in high larval mortality upon bacterial infection and was associated with a high uric acid concentration in larval hindguts. Percentage of larvae reaching adulthood on high dietary P level was significantly lower and was delayed compared to the two lower dietary P levels. Interestingly, larval biomass was similar for the three P-levels. Our study clearly indicates that provision of a high dietary P level in the insect diet not only inhibited development to adulthood but also negatively affected larval health in terms of accumulating the toxic compound uric acid in their gut and therefore adversely affecting their welfare.

Enzymatic catalyzed oxidation in black soldier fly larvae ingredients: identification and activity evaluation of key enzymes

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Fat oxidation is a critical quality concern due to its significant impact on feed quality, palatability and animal health. Multiple factors, including the presence of oxygen, thermal processing conditions, total fat content, and fatty acid composition, can initiate fat oxidation. In the case of ingredients derived from black soldier fly larvae (BSFL), enzymatic catalyzed oxidation (ECO) could also be a major underlying cause. Upon mincing the larvae, released enzymes, particularly lipases, have the potential to contribute to or trigger oxidation if not adequately deactivated. However, there is a current lack of scientific literature on ECO in insect-based ingredients. Hence, this study aims to first identify and assess the key enzymes responsible for catalyzing oxidation and subsequently evaluate their activity in BSFL ingredients after undergoing pasteurization.

The study evaluated the activity of lipase, lipoxygenase, and peroxidase in three BSFL ingredients that underwent through the same pasteurization treatment: minced larvae, protein meal, and fat. The enzymes' presence was measured using; cellulose strips for lipase, horseradish peroxide (HRP assay) for peroxidase and transient free radicals produced by lipid peroxidation (EPR technique) for lipoxygenase.

The results demonstrated that these enzymes were inactive in all products, indicating that ECO is not a causative factor for oxidation.

The versatile microbiome of *Hermetia illucens* guts and frass and its beneficial functions

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Insects play an increasingly important role as alternative source of protein for the sustainable production of food and feed. The Black soldier fly (*Hermetia illucens*) larvae (BSFL) can replace soy and fishmeal in livestock farming and aquaculture. Furthermore, it has been shown that BSFL frass (feces and residual feed) is a potent bio-fertilizer. However, the economic competitiveness of farmed insects depends on the availability of inexpensive insect feed. Industrial side-streams could play a low-priced role in providing feed sources.

Hermetia illucens is a generalist and can be reared on a variety of different waste and side-streams. The degradation of such diverse substrates is mediated by an abundant gut microbiome. We analyzed the gut and frass microbiome of BSFL fed different side-streams via culture-dependent and culture-independent (amplicon sequencing) methods in order to evaluate microbial adaptations and to screen microbes for their functions. We found pronounced changes in gut microbial composition depending on diet, e.g., in larvae fed high-fiber side-streams we found many microbes with cellulolytic activity. During the microbial breakdown of fiber (e.g., cellulose and pectin) methane can be formed and some insect species are already known to emit methane. Therefore, we checked BSFL for methane emission by gas chromatography and analyzed methanogenic archaea in the gut microbiome. We did not find methanogenic archaea in BSFL guts or frass and BSFL did not emit any detectable amounts of methane, indicating that the diets used in this study do not trigger methanogenesis.

Besides the gut microbiome, also the frass microbiome showed differences in microbial diversity and frequency depending on diet. Since some of the detected microbes are known to promote plant growth e.g., the nitrogen-fixing genera *Achromobacter* and *Stenotrophomonas*, there is the possibility of varying the fertilizer properties of BSFL frass by diet.

How can *T. molitor* be implemented as a sustainable source of protein in Austria

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Since many years not only scientists are aware, that a growing population and a rising demand for animal protein could become a huge challenge for the future of mankind. In the r & d project sustainable protein – integrated insect innovations (spi³) a holistic approach was chosen to understand the innovation environment for insects as food and feed in Austria and also to enable innovations. After a market potential analysis and an in-depth analysis of available feed for *T. molitor* feeding trials have been conducted. Mass rearing and sorting facilities have been constructed and built. Mealworm based food products have been developed and assessed concerning consumer acceptance. Analytical assessments concerning amino acids and fatty acids were conducted. In addition, legal and sustainability aspects have been considered overarching the complete value chain.

The holistic r & d approach that integrated the entire food and feed value chain of *T. molitor* helped to understand not only the technological and analytical barriers and opportunities but also the very important societal aspects.

Sex determination in *Tenebrio molitor* beetles is difficult and inconsistent

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Since 2021, *T. molitor* is allowed as food in the EU. Large-scale production operations have been successfully established. As a result of industrial production, breeding efforts are under way to generate beetles and larvae with desired properties, like rapid growth and different preference for feed and water.
However, selective breeding of *T. molitor* is rather difficult as the difference between the sexes is rather small and hard to detect for humans. As we want to mate insects with the desired qualities, it is important that we first identify the individuals, best as pupae, but latest as adults before they start mating. Two methods of sex-determination in adult *T.molitor* have been described in Bhattacharya et al. 1970 and are still in use. Eversion of genitals by exerting gentle pressure on the abdomen is described to be accurate but leads to increased mortality in the beetles when too much pressure is applied. The alternative method, checking the morphology of the membranes between the fourth and fifth abdominal sternites is less intrusive and leads to less mortality. However, both methods are time-consuming and require magnification. The objective of this contribution was to assess the efficiency of both methods.
100 adult *T. molitor* were taken and grouped in batches of five. Five panellists previously trained in both methods sexed all the batches, using either the eversion or the membrane morphology method, i.e. no panellist sexed the same batch twice with the same method. Results were analysed as sets from each panellist for each method and compared via Student t tests. As the sets derive from the same population, it was expected that the results would show a significant correlation among panellists, but no correlation among sets and to a set of random numbers was found. Another
100 adults were left untreated (control group) to assess differences in mortality due to handling. No excess mortality occurred in the group handled by the panel.
In conclusion, sex determination of meal beetles with the current methods varied significantly among panelists and can therefore not be used to reliably tell male from female beetles. However, they were safe to use in terms of animal survival. Better methods, e.g. either visually or chemically need to be developed.

Feed conversion of salad roots in *Tenebrio molitor* across larval development

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The omnivorous nature, high protein content and efficient growth make mealworms an attractive candidate for environmentally-closed ecosystems. As a first step towards their integration, hydroponically cultivated salad roots were identified as a potential feed. This study presents the evaluation of the feed conversion on different proportions of salad roots with wheat bran in different stages of their development.
Freshly hatched larvae were kept on wheat bran and raised to a weight of approx. 15 mg per larvae, accustomed to the experimental diet components and starved. These larvae of similar age were then fed with a new and crude but effective form of controlled feeding in order to minimize feed residues while preventing impairments in larvae development. Feed conversion was measured in approximate digestibility (AD), efficiency of conversion of ingested food to body substance (ECI) and efficiency of conversion of digested food to body substance (ECD).
High ingestion rates could be achieved on all diets with no significant differences in dead and cannibalized larvae per day. ECI and ECD significantly decreased for all diets as larvae grew in weight until first pupae emerged. AD remained mostly constant. Protein content increased (56 to 73%), fat content and dry mass of larvae decreased (34 to 5% and 36 to 20% respectively) between dry mass proportions of 20 to 80% salad roots in the diet.
The decreased efficiency of conversion of ingested and digested food to insect biomass suggests that in order to optimally convert feed, larvae are not required to be grown close to pupation. Although all diets resulted in growth, higher proportions of salad roots re-sulted in detrimental effects regarding feed conversion and growth speed as well as major changes in nutritional composition. Thus, in order to fully substitute wheat bran and con-sequently integrate mealworms in environmentally-closed ecosystems, diversification in diet components will be necessary.

Exploring the potential of black soldier fly-composted frass fertilizer in the control of nematodes and boosting potato yields in Africa

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Potato production in Africa is highly affected by low soil fertility and nematode infestation. The use of mineral fertilizers and synthetic nematicides remains unsustainable due to high costs and negative impacts on humans and environmental health. This study assessed the potential of black soldier fly-composted frass fertilizer (BSFCFF) in the management of potato cyst nematodes and enhancing potato yield under open field conditions.

The experiment consisted of eight treatments: BSFCFF, commercial organic fertilizer (SAFI), BSFCFF + 5% chitin, NPK + commercial nematicide, 50%BSFCFF + 50% NPK, 50%SAFI + 50%NPK, 50%BSFCFF+ 5%chitin + 50%NPK, and Control (unfertilized soil). Data was collected on potato yield, number of cysts/g soil, eggs/J2/g soil and number of eggs/J2/cyst.

Results revealed that soil amendment with BSFCFF, BSFCFF+5%chitin and 50%BS FCFF+50%NPK significantly increased the tuber yield by 170 – 204% compared to the control. The marketable tuber yields obtained using BSFCFF, 50%BSFCFF + 50%NPK and BSFCFF+ 5% chitin were comparable to the value achieved using SAFI . It was noted that all BSFCFF treatments significantly reduced the number of cysts/200g soil, number of eggs/J2/cyst, and number of cyst eggs/J2/g soil by 74 – 117%, 14 – 89% and 41 – 151%, respectively, compared to SAFI. Over 6% reduction in the number of cysts/200g soil was achived using BSFCFF+ 5% chitin when compared with nematicide. Our findings demonstrate the potential of BSFCFF as a multipurpose and regenerative organic fertilizer that could be used by farmers to increase potato yield and suppress potato cyst nematodes across Africa and beyond.

Quantifying dioxins and polycyclic aromatic hydrocarbons in edible insects from East Africa

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Insects are considered highly nutritious and environmentally friendly, although there is a risk that they and their products may be contaminated by environmental pollutants during rearing and processing. The presence of hazardous organic chemicals such as dioxins and polycyclic aromatic hydrocarbons (PAHs) should be investigated to ensure safe product consumption. Grasshoppers, termites, crickets, and black soldier fly larvae were purchased from insect traders in Kenya, Uganda, and Tanzania. Black soldier fly larvae served as a representative matrix for method validation, including determination of limits of quantitation (LOQ) and limits of detection (LOD). For dioxin analysis, 13C-labeled congeners were added to the samples prior to extraction, and the sample was purified using MIURA's GO-xHT system. For PAHs analysis, samples were extracted by energy dispersive guided extraction, and extracts were purified by gel permeation chromatography to separate lipids and proteins from PAHs before sample concentration. The purified extract was measured by high-resolution gas chromatography and high-resolution mass spectrometry (HRGC-HRMS) and quantified by isotope dilution technique. The lipid content ranged from 4.1 to 41.3% for the different species studied. Polychlorinated dibenzofurans (PCDD/F) ranged from 0.03-0.13 pg/g wet weight (ww) WHO-TEQ ub, total dl-PCBs from 0.2-1.0 pg/g (ww) WHO-PCDD/F-TEQ ub, and for non-dioxin-like polychlorinated biphenyls (ndl-PCBs) from 0.03-10.86 ng/g insect sample at 11% moisture content. PAHs analysis is ongoing. Dioxin contamination varies by insect sample and insect species and is generally low compared to other animal protein sources. None of the samples tested exceeded the respective legal limits in the European Union.

Effects of Gainesville diet fortification with steamed legumes on growth performance of black soldier fly larvae compared to chicken feed

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The Gainesville diet (GD) and Chicken Feed (CF) are often used as control diets for black soldier fly larvae (BSFL) fattening experiments. Four GD based diets enriched with protein and energy by steamed legume seeds were compared for BSFL growth performance.

The diets (30 % DM) were composed of 40% wheat bran, 15% maize, 20% alfalfa and 25% autoclaved lentils (LE), peas (PE), beans (BE) or broad beans (BB). The calculated crude protein (CP) and energy (ME) contents ranged from 186 to 199 g CP / kg DM and 9.7 to 9.9 MJ AMEn / kg DM. The basal GD (168 g CP / kg DM, 8.7 MJ AMEn / kg DM) and CF (209 g CP / kg DM, 12.5 MJ AMEn / kg DM) served as controls. On day (d) 5 post hatch, 150 BSFL were placed in rearing vessels (520 ml, mesh window lid, n = 6/ diet, N = 36) that had been filled with 114 g FM diet 24 h previously and maintained at 27.5 °C and 70 % humidity. At d 17 the BSFL where washed, dried, weighed, counted, pooled and frozen (-20 °C). Diets and BSFL were analysed for carbon (C) and nitrogen (N) contents. The C:N ratio, the diet CP content and the non-protein N corrected larval protein (factor 4.76) was calculated. Data of individual larval weight (ILW), frass weight (FW), numbers of larvae (NL), prepupae (NP), mortality (MOR) and feed conversion rate (FCR) were analysed in SAS 9.4 by Proc Anova.

The ILW in the diet groups (LE 108.0, PE 106.8, BE 105.5, BB 93.1 mg) was higher and lower than in GD (65.8 mg) and CF (200.2 mg) controls ($P<0.05$). The NL:NP differed between CF (120:25) and BB (27:128) while MOR was below 2.2 % on all diets ($P<0.05$). The FCR of the legume diets (3.0 - 3.4) differed from CF (2.2) and GD (4.7). The pooled larval C:N was lowest for GD (5.6), higher for BB, BE, LE and PE (5.9 – 6.3) and highest for CF (8.3). The pooled BSFL protein contents in CF (30.9) and GD (35.9) were lower ($P<0.05$) than in legume diets (BB 39.3, LE 38.3, PE 38.1, BE 37.1).

The different legume species in the fortified GD diets similarly improved BSFL growth compared to GD, presumably due to the higher protein, starch and lower fibre content. The higher C:N of CF larvae could be due to the high lipid content, which reduced the relative protein content. The ILW in the fortified diet groups did not reach that of CF.

Advancing black soldier fly selective breeding through computer vision-based phenotyping

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Larval size and weight are important traits in Black Soldier Fly (BSF) production, including selective breeding. Manual measurement of these traits for management decisions and selective breeding is time-consuming and limits the scale of production. Computer vision has increasingly shown to be a promising technology for automatic measurement and monitoring of different livestock traits including growth and health performances. In BSF production, the increasing scale of production and the need for selective breeding to improve larval size and other traits makes the adoption of automation processes essential. In this study, we developed a computer vision system for the automated measurement of BSF larval size and weight. Using a Raspberry Pi module and a Raspberry Pi HQ camera, a data acquisition system is set up with controlled illumination to capture the images of the larva. The system consists of elements for larva counting, size measurement, weight prediction and larval sex prediction. The larval phenotyping element enables measurement of size and weight both at individual and group level (the distribution of size and weight in a tray) from the images. The elements are developed using the deep learning YOLOv8 segmentation module with image processing applied in computing the size and weight of each detected larva. The system allowed detection and counting of BSF larva with 98% accuracy whilst predicting the size of individual larva (body area) with 90% accuracy. This study demonstrates the potential of computer vision and deep learning to improve the efficiency of BSF production and enables scaling of BSF production. The system could be used to select larger larvae for breeding, monitor larvae growth and track trait differences across populations as well as in different rearing conditions including substrates.

Safety assessment of black soldier fly larvae reared on food waste

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The black soldier fly (*Hermetia illucens*) larvae (BSFL) industry currently focus on protein production. In order for it to become a valuable waste management technology linked to protein production in high volumes, the variety of wastes used as feed substrate must be legally expanded. Wastes can be contaminated with pathogens, and lab-scale studies have suggested that BSFL is able to inactivate bacteria, viruses and other contaminants. This study evaluated the inactivation of pathogens (*Salmonella* spp., *Escherichia coli* and bacteriophage φx174) spiked into post-consumer food waste (FW) (up to 10^6-10^7 CFU or PFU g^{-1}) during BSF larvae bioconversion in a large scale (industrial) setting (12,000 larvae in 2400 cm^2 boxes). Three treatments done in triplicates were included in the study: a control with no larvae (CT), one in which the feed load (10.5kg of FW) was provided in two days (T2F), and one in which the feed load was divided into three feeding events (T3F). FW was contaminated with five strains of *Salmonella* spp., three strains of *E. coli* and the coliphage. The experiment was conducted in a controlled environment (31.3±3.1 °C and RH 30.3±4.0%) and lasted for 11 days. The inactivation of *Salmonella* spp. (without differentiating strains) was > 6.4 log_{10} in T2F and T3F, while the concentration remained at 1.2x10^5 CFU g^{-1} in CT. *E. coli* was reduced by > 2.4 log_{10} in T2F and T3F, while the concentration in CT was 1.0x10^5 CFU g^{-1} after 11 days. Coliphage was not reduced at all in the CT, while in T2F and T3F significant reductions were verified (2.20 and 1.73 log_{10}, respectively). The bioconversion efficiency was 22.9±1.0%$_{DM}$ in T2F and 26.2±0.4%$_{DM}$ in T3F, while the material reduction ranged between 65.1 and 71.5%$_{DM}$, respectively. Larvae reached a maximum size of 234 mg in T2F and 179 mg in T3F, with a lower survival registered in T2F (65.3±2.0%) in comparison to T3F (92.0±0.8%). As little or no change of pathogens concentrations were seen in CT, the inactivation observed in T2F and T3F was attributed to larval activity. The material was not yet pathogen-free at the end of the treatment, with 2-6 CFU g^{-1} of *Salmonella*, 1-3x10^3 CFU g^{-1} of *E. coli* and 1-3x10^3 PFU g^{-1} of the coliphage.

Cuticle-reduced black-soldier-fly-meal shows casein-resembling nutritional efficiencies and an appropriate dietary-indispensable amino acids score in mice

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Black soldier fly larvae (*Hermetia illucens*, BSFL) are a sustainable feed protein source due to their complete amino acid profile. However, little is known about their ability to support muscle growth in laboratory mice considered animal models for human nutrition. Additionally, the high cuticle level in BSFL-based meals, mainly from Instar 6, may adversely affect the digestibility and other characteristics of BSFLs' protein quality. We manufactured a low-fat high-cuticle meal from Instar 5 and 6 BSFL and a low-fat low-cuticle meal from Instar 6; and examined their digestibility and protein retention efficiency in four-week-old mice. Compared to the low-fat Instar-5 meal, the low-fat Instar-6-based meal showed higher protein content (50% vs. 41%) yet higher fiber-cuticle content (37% vs. 22%). Given their high fiber content, these BSFL-based meals could replace only 37% of mice's dietary protein (i.e., casein). Mechanically reducing the amount of cuticle in the BSFL6-based meal increased its protein content to 57% while lowering the cuticle level to 14%, allowing for preparing a mixed-protein diet and a diet based solely on this BSFL6-based meal. Compared to the casein-based control diet, all BSFL5/6-based diets showed similar body weight, body composition, food intake, and energy/protein-intake-to-body/muscle weight conversion efficiencies. However, true protein digestibility was lower in cuticle-rich diets than in casein-based diets (94% vs. 98%). Reducing cuticle level improved protein digestibility to 97% in the mixed protein diet and 96% in the solely BSFL6-based diet. Both cuticle-reduced diets exhibited higher retention efficiency of dietary protein to lean body mass than cuticle-rich diets (0.54 g/g vs. 0.39 g/g), suggesting that chitin reduces protein quality or that the cuticle-based protein has lower nutritional quality than the non-cuticle protein. All BSFL-based diets showed a dietary indispensable amino acid score exceeding 100%, hence are suitable for human feeding at all ages. These results highlight the potential of BSFL meal, particularly low-cuticle meal, as an alternative human dietary protein.

How to convince consumers to eat insects?

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In comparison to meat, edible insects are just as rich in nutrients and might even have health benefits. Besides, they are more sustainable, emit fewer greenhouse gases and water and require less land than all other meat products. Apart from their allergenic potential, a number of insect products have been declared safe to eat by the European Food Safety Authority (EFSA). Considering all these benefits why are consumers so reluctant to eat insects? In tropical countries, harvesting and eating insects is often considered a primitive habit, and a habit of poor people. In western countries, the reasons not to consider them as food are predominantly disgust (an emotional rejection response) and neophobia (reluctance to consume unfamiliar food). In Europe only a quarter of the population is willing to consume them. Worldwide the willingness to eat insects often has a cultural background. For example, mealworms were less accepted as food in an insect-eating country like Thailand than in the Netherlands. This because in Thailand mealworms as food were unknown and associated with maggots in carcasses.

The strategies to convince consumers to eat insects are: 1) stress the nutritional benefits; 2) let reliable authorities (such as EFSA) indicate that the products are safe to eat; 3) process the insects into an invisible form in familiar products (although that does not work for sensation seekers who want to see the whole insect); 4) give people a taste experience by organizing bug banquets; 5) provide information, not only about the benefits of eating insects, but also about the way they are prepared; 6) stress the sustainability of the product although it should be realized that this will only have a limited effect; 7) use role models, such as scientists, relatives, and the government; 8) target specific groups such as adventurous eaters; 9) market them appetizing and offer insect-based product in supermarkets.

Consumer studies dealing with edible insects are very new: Three quarters of all publications are from the last five years. Often the reluctance to eat insects is psychological as insect-labelled food not containing insects are often rated inferior. It is expected that the neophobic reactions will diminish rapidly as the information about edible insects is rapidly increasing. Disgust can be lowered by providing as much information as possible about the insect product.

Black soldier fly larvae's role in transforming blends of dairy manure and soybean curd residue mixtures

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The growing numbers 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 fiber 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. 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.

Amsterdam residents' attitudes towards various scenarios of urban initiatives with edible insects

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Edible insects are promising contributors to the circular economy due to their ability to transform low-value organic side streams into highly nutritious biomass. The potential of the introduction of edible insect production in the urban environment is a caveat in academic literature. Taking into account that city residents can be active participators in urban agriculture initiatives, it is essential to understand their opinions and intentions to not only become involved in insect production but also to consume processed edible insects.

A survey was conducted with 750 residents (18-75 years old) of Amsterdam, the Netherlands. The results revealed that Amsterdam residents have little experience in consuming foods with edible insects and whole insects with three-quarters of the respondents having no experience in tasting insects. Also, Amsterdam residents showed various attitudes towards proposed hypothetical scenarios of insect production in households, neighborhoods (centralized facilities for neighbored households), and on an industrial scale. The appreciation of all insect production scenarios was generally low and varied from 6.7% to 24.9% of residents who found insect production in households and on an industrial scale appealing, respectively. The effect of the drivers (safety, expert support, environmental, economic) and barriers (lack of knowledge, lack of available information, economic reasons) was determined and differed for each production scenario. Also, the respondents indicated that the source of insect feed is important for making a decision toward insect consumption.

Thus, this study demonstrated that the ideas of insect consumption and insect production as a part of the urban agriculture concept are still novel for Amsterdam residents. The obtained results can be used in the development of urban initiatives with edible insects to enhance their success and implementation.

Effects of common regional by-products on performance and nutritive value of black soldier fly larvae (BSFL) in fattening

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The need to develop new value chains in a resource-efficient bioeconomy is substantial. Black soldier fly larvae (BSFL) are a promising beneficial insect due to their efficiency in converting organic matter streams into high-value protein.

The study included 420,000 6-day-old BSFL (madebymade GmbH, Pegau, Germany) which were reared in a controlled environment climate chamber for 12 days. The larvae were randomly assigned to four different by-product based diets and one control diet (14,000 BSFL/feeding unit; 6 replicates/ treatment). The diets (group 1: 50% vegetables and 50% pomace, high fiber, HF; group 2: 60% rapeseed press cake and 40% wheat stillage, high protein HP; group 3: 60% old bread and 40% biscuit meal, high Non-structural carbohydrates, HNSC; group 4: BSFL mixed diet of HF, HP, HNSC, Binger diet and group 5: broiler feed as a control (CON)) were adjusted for water content (75%) and offered at 0.2g DM/larvae. Every four days, 30 BSFL/feeding unit were collected, frozen, dried and weighed. Samples of the larvae were also collected.

After 8 days of fattening, BSFL fed the mixed diets showed significantly higher dry matter body weights compared to HF-fed BSFL (BW14: Binger diet=77mg/larvae; CON=80mg/larvae; HF=29mg/larvae; ↑60%; $p<0.001$), while the BSFL fed with HNSC developed the highest weights at 18 days of age (BW18: 69mg/larvae). 18-day-old BSFL fed on Binger diet showed the highest crude protein content, while 18-day-old BSFL fed HNSC presented the lowest protein content (CP18: Binger diet=50.3g/kg DM; HNSC=46.4g/kg DM↑8%; $p<0.05$).

Insect larvae (black soldier fly larvae in this case) also show a special need for nutrients in certain life stages. Classical feed optimization delivers similar performance and represents a bioeconomic alternative to high-quality BSFL feeds (broiler fattening feeds) used so far.

The effect of processing on nutritional value of insects: the case study with mealworm and Jamaican field cricket

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Nutritional value of insects has been described in many papers, but the data on effect of processing on chemical composition are still insufficient. Therefore, the aim of our research was to determine the nutritional composition of culinary treated edible insects with emphasize on quality and quantity of proteins and lipids. *Tenebrio molitor* larvae and *Gryllus assimilis* adults were killed by freezing or by blanching and then culinary treated via cooking in boiling water, roasting, oven drying and microwave heating. Dry matter, ash, total fat, and crude protein contents were monitored, as well as amino and fatty acid profiles and acrylamide levels.

Our research showed that the way of killing and culinary treatment both influenced the chemical composition of the tested insects. The most significant changes were observed in the quantity of nutrients, and fatty acid profiles. In case of *T. molitor* the biggest differences in crude protein content were between microwaved (60.01 g/100 g DM) and boiled (54.76 g/100 g DM) larvae killed by blanching. Total fat content was the highest in boiled (35.28 g/100 g DM) and the lowest in oven dried samples (24.8 g/100 g DM) killed the same way. In *G. assimilis* the highest protein content was in dried blanched (72.95 g/100 g DM) and the lowest in boiled (64.36 g/100 g) samples killed by freezing. These samples had the highest fat content (23.77 g/100 g DM), while the lowest had the dried crickets killed by blanching (13.31 g/100 g DM). The relative proportion of PUFA was increased by all culinary treatments, while the SFA percentage decreased. Unlike the lipids, the quality of proteins was not so affected by the processing. Methionine was calculated as the limiting amino acid. Acrylamide was not detected in the raw samples, but its low levels (0.02-0.13 μg/g DM) were found in the treated samples.

As the raw insects are eaten very rarely, the information about the nutritional value of culinary prepared insets is highly valuable. Moreover, the future research focused on risk compounds connected with thermal treatment and their reduction in insect-based food will be also relevant.

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Small-scale estimation of greenhouse gas emission from insects: the case of black soldier fly larvae on chicken feed

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Edible insects are considered a sustainable alternative to conventional protein. Although several studies have shown that greenhouse gas emission from insects are low, they are usually recorded in closed static chamber, which did not simulate the actual rearing procedures. When open respiration chambers are used, high amount of substrate and insects are needed, making the procedure time consuming and expensive. A novel gas emission device for small-scale detection of greenhouse gas emissions from insect rearing was designed. The device consisted in four independent respiration chambers, each equipped with a pneumatic pump responsible for pumping air inside and a silicon pipe terminating with a flow meter as outlet. A sampling port was inserted along the outlet pipe and sampling was conducted through a gastight glass syringe. Concentration of CO_2, N_2O and CH_4 in the exhaust air was determined by gas chromatography. In order to validate the novel device, an experiment consisting in three independent run and two replicates was performed. Two-hundred black soldier fly larvae and 300 g of moistened chicken feed (dry matter 40%) were used. Rearing was carried out for 10 days with gas sampling every 12 hours. Amount of emitted gas (mL/h) was computed by multiplying the measured gas concentration by the relative airflow. Data interpolation considering a linear trend between two successive sampling points was applied and overall amount of gas was quantified through the area under the curve. Overall, an increase of the CO_2 emission was detected on the complex insects-substrate during the first 7 days of the experiment, followed by a fast decreasing trend. Multiple cross correlation analyses showed high data reproducibility, while significant difference was observed in CO_2 emission (accuracy: 45.47 ± 25.40 ppm) between insects-substrate complex and the substrate itself. N_2O (accuracy: 0.10 ± 0.04 ppm) and CH_4 (accuracy: 25.96 ± 11.94 ppm) could not be detected in the samples, confirming that black soldier fly larvae do not produce such greenhouse gasses. Overall, this experiment showed potential for the novel device to be implemented for fast and reliable estimation of greenhouse gas emissions from edible insects.

Iso waste-based diets as substrate for yellow mealworm larvae

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Yellow mealworm usually grows on dry substrates derived from cereal production, which already have an application in feed industry. The study aims to evaluate the effects of agro-industrial waste and by products combined in iso-nutrient (gross energy, crude protein (CP) and ether extract (EE)) formulations on larval growth and chemical composition. In addition to the control diet (C, wheat bran), 3 dietary treatments were tested (5 replicates/treatment; 10000 larvae/replicate). Waste-based diets were mainly composed by feed and breading waste (FW and BW, respectively) as follows: (TM1) FW and BW + wheat groats, wafer dough, dry stillage and silvery film, (TM2) FW and BW + wafer dough and panettone waste and (TM3) FW + BW + rice by-products. The trial started with 3-weeks-old larvae (WOL) that were weighted every week until the end of larval growth, which was considered when the difference in percentage of the weight between two sampling times was equal or lower to 50%. At the end of the trial, the biomass was weighed and the total number of larvae was estimated. To determine the dry matter (DM), CP, EE, ash and chitin content, larvae were freeze-dried. Data were analyzed by One-way ANOVA (SPSS, v28.0). Until 6 WOL, no difference in terms of growth were observed ($p>0.05$). At 7 WOL, TM1 larvae showed the best growth ($p<0.05$), followed by TM2-3, which performed similarly ($p>0.05$). The C diet displayed the worst growth ($p<0.05$). At 8 and 9 WOL, the growth patterns were the same, as TM3 and C larvae were the heaviest and lightest ($p<0.05$), respectively. The TM1-2 groups were similar ($p>0.05$) and intermediate between TM3 and C ($p<0.05$). At 10 WOL, all treatments were statistically different and, in the weight-scale, were positioned as follows ($p<0.05$): TM3 (0.127g), TM2 (0.118g), TM1 (0.113g) and C (0.087g). As concern the chemical composition, the C group showed lower DM and EE, as well as higher CP and ash content, when compared to the other diets ($p<0.05$) – which were equal in terms of DM, EE and CP ($p>0.05$), while ash was higher in TM2 than TM1 ($p<0.05$). In conclusions, despite the larvae being fed on iso-nutrients diets, the growth performance was affected by the dietary treatment, with TM3 diet – composed by FW, BW and rice by-product – displaying the best results.

Inclusion of rapeseed meal in feed for Jamaican field crickets: effects on feed conversion, nutritional value, and metabolome

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Jamaican field crickets (*Gryllus assimilis*) belong among the most promising edible insect species for production as food or feed. To optimize the farming technology and exploit the potential of edible insects, the feed composition is crucial. Crickets are generally artificially reared on the dried substrates, when soybean is used as a major protein component. In this study, we aimed to evaluate the effect of replacement of soybean meal by rapeseed meal, the by-product from oil production. The crickets were provided by dried "chicken feed based" substrate, when 25, 50, 75 and 100% of soybean meal was replaced by rapeseed meal. Excluding fatty acid profiles, the nutritional analysis revealed no significant effect on the chemical composition of sampled insects. Similarly, no significant difference was observed for the feed conversion. Furthermore, UHPLC-HRMS/MS was applied for metabolomic analysis, when PCA and PLS-DA analysis showed that the insect samples were separated according to their feed composition. In combination with correlation analysis, possible markers of feed alteration in cricket biomass were found and identified based on the exact mass, isotopic profile, and fragmentation pattern of the respective ion. As expected, sinapine was identified as a marker of rapeseed. Although this compound might not be harmful, its bitterness can negatively affect the taste of the final product. Samples were also screened for the presence of antinutritive glucosinolate goitrin. This compound was not detected in any of the tested samples and, therefore, the transfer to the cricket tissues was not confirmed. In addition, the transfer of secondary metabolites typically occurring in soya (daidzein, genistein, saponins) was observed. The results can contribute to the optimization of edible insects farming and to the food industry's circular economy functioning.

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WGS sequencing reveals genomic diversity in *Hermetia illucens*

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Insect farming, and specifically the bioconversion of organic material into animal feed, is increasingly making use of the Black Soldier Fly (BSF). However, in comparison to other farm animals, the genetic resources of farmed insects remain poorly characterized. NRGene is a leading molecular genomics company, we utilize our pipelines to develop modern molecular breeding tools for the BSF.

As part of the Israeli BSF consortium, we have generated the first two BSF high-quality PHASED (separate reconstruction of the sequences corresponding to the two copies of each chromosome) chromosome-scale genome assembly (male and female) BSF. These genomes were then used for downstream analysis for population genetic characterization and diversity analysis. To achieve this, we leveraged our proprietary DeNovoMAGIC™ pipeline, which combines Pacific Bioscience long reads with Illumina short reads sequencing to serve as input for the assembly.

To ensure the quality of the assemblies, we conducted various quality-assurance procedures, including the independent BUSCO benchmark v5.4.4, gene collection: diptera_odb10, which specifically assesses the integrity of genic regions, as well as ploidy and zygosity characteristics of the assembled genomes. The BUSCO benchmark resulted in a score of 96.04% for BSF_IL_Male and 96.13% for BSF_IL_Female.

When looking at the mapping coverage of the contigs used for the assembly, we noticed that chromosome 7 (the suspected X chromosome) contigs coverage was half of the other chromosomes which supports the XY dependent sex determination in BSF. Additionally, when comparing our two phased genomes with the un-phased (collapsed) iHerIll genome, we found unmapped scaffolds unique to the BSF IL Male assembly, suggesting that these scaffolds may belong to the previously uncharacterized male Y chromosome. We then used the male BSF_IL_Male assembly as a reference for diversity analysis and populations genetic comparison,sequencing more than 16 population (5 male and 5 females per population). The analysis results illustrate the high diversity that exists between populations from around the world. We also found That the Y related scaffolds, segregate in the population, and exist only in males, validating our hypothesis that these scaffolds belong to the Y chromosome.

Our next step is to generate a genetic SNP panel that harbors this diversity and male-female identification SNPS. These panels will be facilitate the development of advanced breeding models.

What are we missing in our genomes? Revealing the hidden variation in black soldier fly genomes

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The generation of reference genomes has become easier and cost-effective, providing a valuable resource for academia and industry. However, reference genomes are typically derived from a single individual specimen, often from a colony and potentially inbred. Although this facilitates genome assembly, it limits our understanding of genetic variation. When studying insect genomes to correlate phenotypic traits, additional individuals with varying traits are sequenced, and their genetic data is compared to the reference genome. But what if the information needed is not present in the reference genome? How much data are we missing by relying solely on a single genome? This issue is particularly relevant when studying black soldier flies (*Hermetia illucens*), known for their significant variation. Even in industrialized strains originating from a common starting population, such as the Sheppard strain, substantial variation persists. In this presentation, we will showcase data demonstrating the extent of data missed when relying solely on the reference genome for five strains. These strains were sequenced using short-reads, mapped to the reference genome, and the remaining unmapped reads were assembled. Our findings support the argument that pangenomic approaches are necessary to advance the industry's understanding of trait variability and optimize production processes.

Improving black soldier fly genetics by crispr\cas9 gene editing

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The shortage of protein resources is becoming more and more severe as the global population is increasing. Finding new environment-friendly and sustainable protein sources is a necessity to replace the traditional ones. The black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae), is one of the most efficient insects in converting organic waste into biomass. To generate improved genetic strains with beneficial features, we utilized the CRISPRIL platform to develop an efficient BSF genome editing platform. Applying a unique gRNA design system developed during the CRISPRIL consortium, we generated 400 genome editing events with an average efficacy of 74%. Additionally, we generated 10 deletion events by applying CRISPR on two adjacent editing sites simultaneously. Later, this technique was applied to generate modified BSF strains with enhanced industrial performance. One of these strains resulted in significantly larger larvae than the wild-type origin. This strain has a better feed conversion ratio (FCR) while maintaining its nutritional values. Our study provides valuable technical and genomic resources for improving BSF lines for industrialization.

Meeting the insect industry's demands: highlights from a voucher scheme

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Tackling industry questions via a voucher program is one way to offer quick support to (start-up) companies with limited R&D budget. These questions are diverse and often require specific expertise. A selection of cases will be presented related to insect rearing.

A first voucher focused on the set-up of a sustainable cricket (*Acheta domesticus*) farm by implementing pre-consumer waste from retail in cricket feed. Crickets are usually reared on a dry feed that is supplemented with a source of water. This can be offered to the crickets as pure water or as fruits and vegetables. The predominant residues from a local retailer (bread and an array of vegetables and fruits) were identified and tested as dry or wet feed ingredient for crickets.

In a second voucher, a horse feed manufacturer asked to valorise his by-products from the milling process in collaboration with a producer of mealworms (*T. molitor*). The by-products mainly consist of cereals and herbs. Three different cereal based feed treatments were constructed and tested in all life stages of mealworm farming: egg production, growth of neonates during the first few weeks and during mealworm fattening.

In the third voucher, initiated by a start-up, several feed companies were contacted with the question if they were interested to participate in a trial where they got the opportunity to formulate feed for the black soldier fly larvae (*Hermetia illucens*). This feed should be circular, sustainable and meet the requirements needed for black soldier fly production. Several feed formulations, meeting minimal macro-nutritional requirements, from six different companies were tested for black soldier fly larvae production.

Ammonia emissions of *Hermetia illucens* larvae grown on different diets

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Ammonia emissions and their impact on eutrophication, particularly in relation to intensive livestock farming, have led some countries to implement measures to mitigate these emissions. However, the emergence of a new activity involving the production of black soldier fly larvae could potentially contribute to ammonia emissions. While existing studies have explored this topic, the wide range of potential diets and variations in rearing techniques necessitate further research to enhance our understanding in this area. This research is crucial to provide the knowledge required by local decision makers to support the establishment of new black soldier fly farms.

To investigate ammonia emissions, a study was conducted using an accumulation chamber - a stainless steel closed box that measures the concentration of ammonia. Within this chamber, a crate (60 cm x 40 cm) containing growing larvae (15,000) and their feed (10 kg) was placed for a short period (ranging from 10 minutes to 1 hour) every 24 hours. By fitting an exponential function ($f(t) = a*(1 - b * \exp(-ct))$) to the ammonia evolution in the accumulation chamber and deriving it at time zero, the instantaneous ammonia emitted by the crate at the time the larvae were introduced could be determined. The combined data obtained throughout the larval growth phase allowed for the identification of peak and total ammonia emissions. This methodology was applied to five different diets: Gainesville diet, chicken feed, swill, and brewer's spent grains. Furthermore, protein levels of swill and brewer's spent grains were adjusted to provide insights on the influence of protein on ammonia emissions. Diets were either enriched with casein protein or had protein levels diluted using beet pulp or hay pellets to achieve varying protein content, specifically: pure diet, 12% crude protein on a dry matter basis, 18%, and 25%.

In addition, the nitrogen balance was determined through chemical analysis of the feed, larvae, and frass (excrement), while the pH and temperature of the substrate inside the crate were monitored. The combination of these parameters allowed for a comprehensive understanding on ammonia production in relation with time.

Implementing mealworm oil in cosmetics

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The implementation of insects in food and feed is growing. Of particular interest are mealworms (*Tenebrio molitor*) as they are amenable to large scale farming under sustainable conditions. When implemented for food and feed, mealworms are often processed, thereby separating the oil from the other fractions (protein and chitin). The resulting oil is a by-product which can be used in other applications.

Here we describe the extraction and characterization of mealworm oil and its implementation in a cosmetic hand cream, body cream and body oil formulation.

Mealworms were killed by blanching (40sec) and were then oven-dried (65°C). The dried mealworms were grinded and oils were extracted with ethyl acetate. Typical yields are 20-30% of the dry weight. The oils were further refined by steam distillation followed by centrifugation, resulting in yellowish oils with low free fatty acid value, low peroxide value and low phospholipid content. The fatty acid profile can vary between batches of mealworms, but in general it is suitable for cosmetic applications. Palmitic acid, oleic acid and linoleic acid comprise >90% of the fatty acid profile. Physico-chemical properties (density, viscosity, surface tension) are similar to currently used oils (eg. mink-, macadamia nut- and almond oil).

When substituting the plant or animal oils with mealworm oil in cosmetic formulations only minor differences are observed. The formulations are equally stable (pH, colour, odour, aspect), they don't interfere with the preservation agents and have similar properties (spreadability, texture). When tested by a professional panel no sensorial differences are observed between formulations containing mealworm oil as an ingredient or formulations with the original plant oil.

Our results indicate that mealworm oil is a novel, sustainable ingredient that can be used in the cosmetics industry.

Evaluation of poultry bloodmeal as a constituent of housefly larvae feed

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Housefly larvae are very rich in nutrients which makes them an excellent substitute or supplement for animal nutrition. They can increase food security when used as a replacement for fishmeal or soybean in the fish and poultry industry. Housefly larvae are easy to grow on various substrates including waste streams. Substrate composition is an important factor affecting the growth and weight of housefly larvae. There is a strong need to further explore the suitability of various kinds of feed for rearing housefly larvae that are sustainable and economical.

Dried poultry blood is a potential substrate for housefly production. It contains about 95% protein and some other micronutrients. We investigated the suitability of poultry bloodmeal as feed ingredient in the rearing of housefly larvae and its effect on the growth and weight of the larvae. Different proportions of the standard medium consisting of wheat bran, inactivated yeast, wheat flour and water were replaced by poultry bloodmeal, 0% (control), 20%, 40%, 60% and 100% (pure), keeping the total amount of available food constant.

On the fifth day of the experiment the larvae were harvested to determine their fresh weight (per larva and total larvae), dry weight (per larva and total larvae) and the weight of the rest material. Adding 20% bloodmeal to the substrate yielded larval weight slightly but not significantly lower than the control, whereas 100% of bloodmeal led to larvae with very low weight. As bloodmeal has very high protein content, at high concentration it appears to become limiting in other nutrients such as carbohydrate, to sustain larval growth. This study provides insight into the percentage of poultry bloodmeal that can be used to produce high weight housefly larvae.

Cross-cultural survey on consumer acceptance of insect-based food in China and France

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The growing interest to use edible insects as a food source has recently gained considerable attention due to its beneficial effects on the environment, nutrition, and food security, compared to conventional protein sources. However, despite being a traditional food in certain regions, the acceptance of edible insects remains limited, particularly in Europe. In addition, only few studies have conducted cross-cultural investigations on the acceptance of edible insects.

This study aims to explore consumers' willingness to consume insect-based foods and examine the factors that determine acceptance in two countries. An online cross-cultural survey was conducted in France (n = 515) and China (n = 594). Multiple linear regression was used to analyze the influence of several explanatory variables on the acceptance of insects as food.

The key finding indicated that Chinese respondents exhibited a higher willingness to consume insect-based food products compared to the French respondents, especially products including whole insects. Moreover, factors such as reduced visibility of insects, low food neophobia and disgust, prior experience with entomophagy, and knowledge about the benefits of insects (e.g. nutritional value and environmental benefits) were significant predictors of consumers' disposition to consume insects in both countries. Therefore, incorporating insects into the daily diets of French and Chinese consumers requires a transitional phase, wherein consumers become accustomed to processed foods containing unnoticeable insects and develop a positive perception of insect-based food, which is more likely to lead to successful acceptance.

Live insect larvae and meat quality in broiler chickens: can the different cooking methods influence meat proximate composition?

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Cooking methods have been reported to influence nutrient composition of meat and fish, but no information is available in poultry. Since insect-based products may affect meat quality in broilers, this study investigated the effects of *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) live larvae as environmental enrichment on proximate composition of cooked meat in broiler chickens. A total of 180 4-day-old male broiler chickens were randomly allotted to 3 groups (6 pens/group, 10 birds/pen): a) control group (C, commercial feed), b) HI group (commercial feed + HI live larvae at 5% of the expected daily feed intake [DFI]) and c) TM group (commercial feed + TM live larvae 5% DFI). At 38 days of age, 2 birds/pen were slaughtered, and right and left breasts were collected. Breasts were divided in three portions: i) cranial, ii) intermediate, and iii) caudal. Cranial and caudal portions were submitted to 4 cooking methods (oven [O], bain-marie [BM], plate [P], grill [G]). Proximate composition (dry matter [DM], crude protein [CP], ether extract [EE], ash) of raw (intermediate portion) and cooked meat was evaluated. Data were analyzed by GLM (SPSS software, $P<0.05$). The P and G cooking led to higher meat DM, CP and ash contents than O and BM ($P<0.001$), thus reflecting the increase in nutrient concentration due to the highest moisture losses. Live larvae administration did not influence the proximate composition of raw meat ($P>0.05$), while, independently of the cooking method, larvae-fed broilers showed decreased ash content ($P<0.001$). Larvae-fed birds also displayed lower meat DM, CP and ash contents than C group when submitted to O and P cooking, with BM decreasing ash content as well. These outcomes were reasonably related to the different nutritional profile and predominant water content of the insect larvae. Differently, no significant differences related to diet, cooking method or their interaction were observed for EE content ($P>0.05$). Cooking methods may influence meat proximate composition in broilers as a consequence of the different moisture losses and nutritional profile of the insect larvae.

Live black soldier fly larvae improve growth performance and feed efficiency in broiler chicken rearing

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Broiler chickens in commercial systems spend much of their time sitting, which can negatively impact animal welfare. An enriching environment, such as insects, which are a natural part of poultry diets in nature, could increase broiler chickens' activity while rewarding their consumption.

To explore whether live larvae have a positive effect on animal welfare, the present study considered the nutritional aspects. For this purpose, the effects of feeding live black soldier fly larvae (BSFL) on performance parameters in growing broiler chickens were investigated. A total of 36 one-day-old broiler chickens (Ross 308) with an initial body weight (BW) of 52g (±2g) were equally divided in two feeding treatments (3 pens of each 6 birds = 18 birds/treatment): The control group (CON) consisted of commercial feeding with broiler fattening diet (basic ration), the experimental group (BSFL) received live BSFL (5% (DM) of expected daily feed intake (DFI)) in addition to the basic ration, until day 21. From day 21, the BSFLs were increased to 10%. BW and feed intake (FI) were recorded weekly to calculate feed conversion ratio (FCR).

After 28 days of fattening, animals on the BSFL diet showed significantly higher BW (BW28: CON=1793g; BSFL=1888g; ↑5%; *p=0.016*) with numerically lower DFI (DFI0-28: CON=89g/day; BSFL=85g/day; ↓4.5%; *p=0.171*), resulting in significant improved FCR of the basal ration (FCR0-28: CON=1.34g/g; BSFL=1.22g/g; ↓9%; *p=0.014*).

In conclusion, feeding 5-10% live BSFL improved animal growth performance of broiler chickens and reduced feed consumption.

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Rare earth elements bioaccumulation in *Hermetia illucens*

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Digested municipal sewage sludge is problematic waste. Classical methods of its disposal involve using as fertilizer for agricultural purposes and land reclamation or it can be added to fuel combusted in incineration plants. The worst solution is to disposal in landfills or in seas. Its production in worldwide is estimated to grow – only in Europe 7 million tons of dry weight of sewage sludge is produced each year. From the other side, research have shown that during the year of operation of municipal treatment plants, significant loads of rare earth elements (REE) passes through them and concentrate in the sludges.

Common biological method of sludge management is vermicomposting, which is the use of earthworms for its treatment. However, in recent years there has been progress in research on the use of saprophagous insect larvae for sludge disposal. *Hermetia illucens* is an insect from the Diptera order, Stratiomyidae family, which can be used for reduction of dry weight of the sludges. The aim of the study was to investigate bioaccumulation potential for REE by *H. illucens*.

In this research we offered digested municipal sewage sludge obtained from “Hajdów” wastewater treatment plant of Lublin and near cities to 7-days-old *H. illucens* larvae. Experiment included also variants with the addition of EDTA as a chelating agent for elements. Potential REE bioaccumulation have been investigated in all developmental stages of the insects and in exuviae. Results showed that most of REE had non-zero concentrations in different stages of the insect, however, bioaccumulation occurred only for few elements and it was: Lu, Tb and, most notably, Dy.

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Insecticidal potential of chitin-enhanced black soldier fly frass fertilizer extracts against onion fly (*Atherigona orientalis* Schiner)

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Bulb onion is among the most consumed vegetables in Sub-Saharan Africa, but its production is significantly constrained by soil-dwelling pests, especially the onion root flies that double as vectors. This study evaluated the insecticidal potential of chitin-enhanced black soldier fly (BSF) frass fertilizer extracts as a sustainable alternative for management of the onion fly pest.

The chitin-enhanced frass fertilizer extracts (ChEFFE) were prepared by fermenting BSF frass fertilizer and different rates of BSF pupae exuviae powder in liquid solutions containing effective microorganism culture and molasses. The insecticidal activity of ChEFFE was investigated using contact, residual and ovicidal assays. Contact assays were conducted by dipping twenty onion fly larvae in ChEFFE for 10, 60 and 300 seconds while residual assays involved feeding larvae on macerated onions mixed with 10 ml of ChEFFE. In both assays, larval mortality was recorded after 24, 48, and 72 hours. The ovicidal assay was conducted by exposing onion fly eggs to the extracts through contact methods and the ovicidal action scored after 72 hours.

Results revealed that the larval mortality increased with inclusion rate of BSF pupae exuviae in the frass fertilizer extracts, exposure time and the number of hours after exposure. It was noted that ChEFFE significantly inhibited eclosion of onion fly eggs by 65% after 72 hours. For the larval stage, the highest larval mortality rate (21%) was achieved after exposure to ChEFFE for 60 seconds, while the lowest value (14%) was recorded after an exposure time of 10 seconds. Yet, prolonging the exposure time beyond 60 seconds did not have a significant impact on larval mortality. In residual bioassays involving first and second instar larvae, the highest mortality rates of 31% and 22%, respectively, were achieved after 72 hours. These findings demonstrate the potential of ChEFFE as a biorational pesticide for management of onion root flies. Effective management of onion root fly using ChEFFE should target the egg stage or first instar larvae.

Insects as feed: antibacterial and prebiotic potential and in-depth characterization of *Hermetia illucens* L. larvae and of their isolated protein, lipid, and chitin fractions

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The sustainability of the animal breeding sector, one of the most impacting in terms of waste production and exploitation of resources, is highly determined by the natural resources used for feed production. In recent years, insect farming has been gaining attention mainly for the insect bioconversion ability for agri-food by-products and their relatively low needs of resources in terms of water, land, and energy. Moreover, insects represent a great source of essential nutrients and of compounds with potential antimicrobial and prebiotic activity, namely lauric acid/antimicrobial peptides and chitin, respectively.

In this context, *Hermetia illucens* larvae were studied as a potential novel functional feed ingredient for swine by testing the antimicrobial and prebiotic activity of the whole insect and its specific fractions. Specifically, the larvae underwent a preliminary defatting step by Soxhlet extraction to isolate lipids and to facilitate the subsequent protein extraction. The protein and chitin fractions were then obtained by Osborne fractionation. The whole larvae and their isolated fractions were then characterized in terms of proximate composition, protein and amino acid profiles, chitin content, and fatty acid distribution.

Then, the antibacterial activity of the larvae and their fractions was tested *in vitro* against *Escherichia coli* F4 and *Streptococcus suis*, two common swine pathogens. Moreover, their prebiotic activity for *Lactobacillus reuteri,* a swine commensal, was also evaluated.

Results showed that *H. illucens* larvae and their isolated protein fraction exhibited antimicrobial activity against *E. coli* F4, while their isolated lipid fraction against *S. suis*. No antibacterial activity was associated to the chitin fraction. In addition, the larvae and their chitin fraction showed prebiotic activity for *L. reuteri*.

From the results it can be inferred that the functional benefits derived by the use of insect fractions as novel feed ingredient might be of interest, as they represent a source of high-quality nutrients and bioactive compounds whose antibacterial and prebiotic activity have been confirmed *in vitro*.

Using insect-composted organic fertilizer to increase yield and economic returns of bush beans (*Phaseolus vulgaris*)

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Black soldier fly frass fertilizer (BSFFF) has gained global prominence due to its potential to enhance soil health and crop production. However, there is limited knowledge about its performance on leguminous crops. This study evaluated the comparative effect of BSFFF and other commercial fertilizers on the growth, yield and profitability of bush beans (*Phaseolus vulgaris* L.) (Rosecoco GLP2).

Both BSFFF and commercial organic fertilizer (phymyx) were applied at rates of 0,15, 30 and 45 kg N ha^{-1}. Inorganic fertilizer (NPK) was also applied at 40 kg N ha^{-1}, 46 kg P ha^{-1}, and 60 kg K ha^{-1}, as positive control. Rhizobia inoculant was incorporated into the experimental set-up to assess its interaction with both the organic and inorganic fertilizers.

Our results showed that the chlorophyll content of *P. vulgaris* grown in plots treated with BSFFF at a rate of 45 kg N ha^{-1} was significantly higher compared to phymyx (commercial organic fertilizer) at equivalent application rates. Bean plants were taller, and had higher number of leaves, flowers, pods and seeds per pod in plots treated with BSFFF and phymyx applied at 45 kg N ha^{-1}, but combination of BSFFF with rhizobia showed no significant difference in bean growth. Application of fertilizers at rates beyond 30 kg N ha^{-1} significantly increased *P. vulgaris* yield compared to unfertilized soil. Bean yield was 12% higher in plots treated with BSFFF rate of 45 kg N ha^{-1}, compared to plots amended with phymyx at equivalent rate. Use of BSFFF increased bean yield by 4 – 34% compared to NPK fertilizer, while combination of rhizobia and BSFFF applied at rates ≤ 30 kg N ha^{-1} increased bean yield by up to 14%. Economically, bean production using BSFFF increased the net income by 1.6 – 9 folds compared to phymyx application.

Our findings show that BSFFF was more effective than phymyx and NPK at improving bean growth and yields, and returned higher profits compared to phymyx. This study provides a scientific basis for selecting novel and effective organic fertilizers such as BSFFF that can replace the conventional organic and synthetic fertilizers used in bush bean production systems.

Insect Farming Innovations: Lessons from Africa

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In Africa, insect farming is a rapidly growing agribusiness, coupled to the rising demand for alternative proteins for food and feed. This review highlights the status and emerging gaps in edible insect farming, role of private sectors, potential estimates; processing, nutritional composition, safety, application, and legislative framework governing the industry. Thirteen (13) edible insect species were identified during this survey producing under farmed and semi-farmed conditions. Over 2300 active edible insect farms are operational but this list is not exhaustive as the number of new entrants, companies and initiatives continue to increase. These farms compromise small, medium, and large-scale private farms. Black soldier fly (BSF) is most popularly farmed edible insect due to its efficiency to recycle a broad range of organic substrates to into multiple high-value market products: oils, chitin/chitosan products, frass fertilizer, protein press cakes (defatted), and briquettes. The production capacity of insect protein by these companies ranges between 6 – 3600 tons of dried BSF larvae per year. Over 90% of livestock and fish farmers, and feed millers are willing to pay and integrate insect protein in their feed. However, not up to half have successfully achieved this. The insect farming industry is highly profitable and the prices for farmed insect products remain high, though becoming more competitive, as the industry matures. Advanced continental regulatory framework is urgently needed. Evidence suggests that although the industry is still in its infancy, this eco-friendly insect-based technology.

Socioeconomic and ecological impact of insect farming in sub-Saharan Africa

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The increasing human population, income growth, and urbanization trends in sub-Saharan Africa (SSA) have led to a consistent rise in the demand for animal-sourced food. Simultaneously, rapid urban expansion produces significant quantities of biowaste, posing ecological and food security challenges. The COVID-19 pandemic, coupled with regional geopolitical conflicts, further disrupted the already delicate food, feed, and fertilizer markets, shedding light on Africa's fragile food system.

Current resources for food and feed production are being exploited to excess while expanding them raises environmental concerns. Insects, such as the black soldier fly, emerge as a sustainable, ecologically friendly solution to address the growing demand for protein and fertilizers and to tackle waste management. Mass-rearing insects transform organic waste into high-quality nutrition for animals and plants, creating job opportunities, enhancing food security, and fostering sustainable agri-food systems. This approach also holds the potential for ecological health in the context of climate change.

Insect framing, although in its infancy as a potentially lucrative agribusiness, requires solid evidence generation to foster scaling and facilitate the transformation of the agri-food systems in SSA. This presentation offers a comprehensive examination of the insect farming industry in SSA, touching on the policy environment and the sector's ecological, social, and economic benefits.

By enhancing awareness among stakeholders, our goal is to stimulate dialogue and encourage more research into how insect farming can strengthen the resilience of agri-food systems in SSA. This is an opportunity to revolutionize food production while dealing with waste management issues and mitigating the impacts of climate change, paving the way for a sustainable future.

Efficiency of garden fruit chafers to recycle animal manure: Implications on fertilizer quality, pathogen suppression and crop yield

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The role of insects as nature-based recyclers have given rise to captive mass rearing worldwide. However, no information exists on transformative ability of garden fruit chafer (*Pachnoda sinuata* L.) to recycle waste into value added products. This study evaluated the nutrient quality, maturity, stability, and agronomic effectiveness of *P. sinuata* composted fertilizer on vegetable production and associated nutrient quality.

The larvae of *P. sinuata* were used to compost cattle dung in four-litre rectangular plastic containers into frass fertilizer (FF). The FF was analyzed for nutrients, maturity, stability and presence of pathogens using standard methods. Comparative performance of FF and commercial organic fertilizer (SAFI) on the production of indigenous *Amaranthus dubius* was conducted using greenhouse experiments. The FF and SAFI were applied at varying rates (0, 25, 50, 75 and 100 kg nitrogen (N)/ ha) to determine the optimal rate for improved amaranth yield and nutritional quality.

Results showed that composting cattle dung using *P. sinuata* significantly increased the concentrations of N, phosphorus (P), copper, sulphur, potassium (K), zinc and boron (B) in the product (FF) by 14%, 20%, 27%, 50%, 64%, 66% and 124%, respectively. Use of *P. sinuata* to recycle the cattle dung significantly reduced ammonium, organic carbon, ammonium to nitrate ratio and C/N ratio as well as pathogens, particularly *Escherichia coli* in the FF within 28 days. Both fertilizer amendments significantly increased the growth and yield of Amaranth compared to unfertilized soil. Soil amendment with FF increased amaranth yield by 48 – 154% compared to SAFI. The N (42 – 129%), P (1.4 – 4.4 folds) and K (1.4 – 6.5 folds) accumulated by amaranth grown in soil amended with FF was significantly higher compared to other treatments. Furthermore, amaranth grown using PSFF had 28%, 28% increase in energy, while the P (17 – 123%), K (3 – 19%), B (14 – 21%), and 78% molybdenum levels were higher, compared to SAFI. Our findings demonstrate the efficiency of *P. sinuata* larvae to recycle low-value organic waste into high-quality and safe fertilizer that can be integrated into existing regenerative agricultural interventions for improved food and nutrition security.

COMPANY PRESENTATIONS

ABSTRACTS

The importance of precision feeding in insect farming

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With evolving feeding strategies and accelerating research related to feeding substrates for different insect types, the need for accurate and fast feeding solutions becomes increasingly important.

The range of allowed feeding substrates is growing as research proves the capabilities and safety of using them. Therefore there is a growing demand for equipment that can handle a wide range of feed substrates dose it in an even and accurate portion .

Many factors are important to take into account when feeding insects, such as particle size, moisture content, free water, fat content, temperature and as well the way it is brought towards the insects.

Accuracy finds its importance in the amount of substrate in relation to the density and amount of larvae in the crates. The feed portions should be delivered in a clean and fast manner, that results in an optimum uptime of the feeding lines, intralogistics systems and harvesting equipment.

Precision however finds its touch points in the way of bringing the substrate into the crates. Examples of this are the layer thickness for BSF substrate and the homogeneous distribution of dry- and wet feed for mealworms.

Rearing efficiency can be improved by taking the insect type as a basis, translate their needs in preparing the substrates in a right manner and dosing this with the right precision. Equipment will no longer be the limiting factor on this subject.

Insect farm automation: the benefits of data

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Farm automation offers benefits beyond simply replacing manual work with more productive machines. In this presentation, Viscon will provide insight in the value created by connecting machines. Also, we present how data from various suppliers can be combined in a pragmatic and meaningful way.

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

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Insect farming and cultivation, particularly of species such as black soldier flies and mealworms, has gained significant attention as a sustainable solution for various industries, ranging from animal feed production to waste management. However, the complex nature of insect farming presents unique challenges in terms of planning and execution. This presentation delves into the intricacies of planning insect farming projects, highlighting the need for intense multidisciplinary collaboration and effective project management techniques.

Insect farming projects encompass diverse sizes and constraints, necessitating tailored planning strategies. Each project is inherently unique, demanding a harmonious blend of biological, engineering, and economic expertise to create a cohesive and adaptable model. The integration of these disciplines establishes a solid foundation for successful project implementation.

The planning horizon for insect farming ventures is extended, further complicated by the dynamic nature of assumptions and constraints. This fluidity underscores the requirement for a hybrid approach, combining traditional waterfall methodologies with agile project management techniques. The utilization of both methods ensures adaptability while maintaining a structured framework.

However, effective planning is only one facet of project success. Stakeholder management emerges as a critical factor in achieving favorable outcomes. Engaging stakeholders, addressing concerns, and aligning expectations are paramount to fostering cooperation and support throughout the project lifecycle.

This presentation not only offers insights into the intricate planning processes involved in insect farming projects but also presents a compendium of best practices. By examining common pitfalls and lessons learned from practical experiences, this paper equips project managers with valuable knowledge to navigate the complexities of insect farming initiatives.

In conclusion, a comprehensive and adaptable planning approach, rooted in multidisciplinary collaboration, is pivotal for successful insect farming projects. By embracing a hybrid project management methodology and prioritizing stakeholder engagement, project managers can forge a path towards successful execution, ultimately ensuring the contentment of all stakeholders involved. This presentation synthesizes the core tenets of insect farming project management, providing a roadmap for prosperous ventures in this burgeoning field.

POSTER PRESENTATIONS (ABSTRACTS)

Incorporation of whole silkworm (*Bombyx mori* L. 1758) pupae meal in rabbit feed: effects on growth and meat fatty acid profile quality

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Silkworm pupae (SWP) are the main by-product of sericulture. The large quantities produced are under-valued. Spreading them on fields is a source of pollution for producer countries. However, SWP are rich in high-quality macronutrients. For several years now, its use as animal feed has been investigated. The environmental challenge is twofold: to make silk production more sustainable and to meet the growing demand for animal protein. The incorporation of whole meal or oil from SWP into the poultry and fish industries has already been the subject of numerous studies.

The production of meat rabbits is also an interesting way of valorizing SWP. Rabbits have a short life cycle and a high conversion rate (+- 20%). Moreover, it produces a good quality meat and its fatty acid profile is partly modulated by its diet. Studies on the incorporation of whole SWP into rabbit feed are scarce. The effects on growth and meat quality remain unclear.

In this context, this study evaluates the effects of incorporating 5% SWP into rabbit diets on growth, carcass characteristics and meat fatty acid profile. A control diet (C) and a diet containing 5% SWP (SW) were fed to two groups of 24 rabbits during the last 3 weeks of life. Formulations were isonitrogenous, isolipidic and isoenergetic.

Final slaughter weight showed no difference between both groups, nor did carcass characteristics apart from heart weight. Meat from rabbits fed 5% CVS contained higher levels of the fatty acids C16, C18:3, C20:5 and less C18:2 than in the control group. In conclusion, SWP is a high-quality source of protein and fat for rabbit feed. It can be incorporated at a level of 5% without deleterious effects on growth or carcass characteristics.

Safety and quality of farmed insects

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INPROFF, which stands for "Insect Protein as Food and Feed", is a three-year project funded by the Slovenian Research Agency (ARRS) and the Czech Science Foundation (GAČR). The project aims to bridge the knowledge gap regarding the quality, safety, and authenticity of farmed insects, as well as to explore strategies for enhancing the value chain of insect-based food and feed production. As part of this work, we have investigated the quality of freeze-dried *Tenebrio molitor*, *Locusta migratoria*, and *Acheta domestica* in terms of fatty acid and polyphenol composition, as well as their safety concerning potential toxic elements and pesticide residues. Fatty acids and polyphenols were determined using GC-MSD and LC-MS/MS methods, while the elemental composition was determined using an optimized ICP-MS method. Additionally, target analysis was employed to identify possible residues of over 300 currently used pesticides, and comprehensive workflows have been developed to facilitate both suspect screening and non-targeted LC-HRMS-based investigations. Furthermore, the potential health risk was also calculated based on the data obtained. Some preliminary results from the project will be presented.

Monitoring microbiological hygiene indicators in silkworm (*Bombyx mori*) farming

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Silkworm (*Bombyx mori*) has recently gained approval for use as animal feed in Europe. Silkworm pupae are the main by-product of silk production and hold significant potential as a versatile option for both food and feed applications. Thus, it is essential to evaluate the microbiological risks linked to silkworm farming, as they can potentially carry microorganisms that may contaminate feed and food. The objective of this study is to assess microbial contamination at various stages of insect rearing and in the rearing environment. We monitored four Italian silkworm farms during spring 2022, collecting both environmental and silkworm samples, the latter from the first instar to dried pupae. Samples were analysed for a limited number of microorganisms, such as mesophiles, coliforms, yeast, and moulds, which are commonly used as indicators of hygiene quality in food production environments.

The microbial load underwent significant changes during the different larval instars, with the highest values observed during the third instar. When comparing different farms, the fifth-instar larvae displayed varying levels of contamination for mesophiles, moulds, and yeasts. However, the microbial load in spinning larvae, fresh pupae, and dried pupae was similar across all farms, with no significant differences detected.

A general reduction in microbial load of dried pupae was observed with respect to fresh ones for both coliforms and mesophiles, indicating the effectiveness of the drying process (80°C for 2 hours followed by 2 hours at 70°C and 4 hours at 60°C) in decreasing microbial contamination.

The study emphasises the importance of maintaining good hygiene practices during the silkworm rearing process. Despite thermal processing can effectively mitigate the risk of microbial contamination, the implementation of proper hygiene measures from the earliest stages of rearing is crucial to minimise the potential microbial risks associated with silkworm-derived products intended for food and feed applications.

Mealworm chitin as asorbent in the removal of reactive dyes from aqueous solutions

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The aim of the study was to test the possibility of using exoskeletons and exudates from mealworm larvae (Tenebrio molitor) as sorbents for anionic dyes: Reactive Black 5 (RB5) and Reactive Yellow (RY84) and cationic dyes: Basic Violet 10 (BV10) and Basic Red 46 (BR46) from aqueous solutions The investigated factors included: characteristics of sorbents (FTIR, pHPZC), effect of pH on sorption efficiency, sorption kinetics (pseudo-first, pseudo-second order, intramolecular diffusion) and determination of maximum sorption capacity (Langmuir 1, Langmuir 2 and Freundlich models).

The efficiency of sorption of anionic dyes on mealworm chitin was highest at pH 2. The time to sorption equilibrium for both dyes was 120-150 min. Data on maximum sorption capacity showed the best fit to the Langmuir isotherm 2, suggesting that at least two types of sorption centers played an important role in dye sorption. Presumably, for both tested sorbents, the active sites in question were protonated amines (-NH3+), acetamides (NH2COCH3+) and hydroxyl groups (-OH2+) of chitin and protein.

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Attitude of owners and animals treated in veterinary clinics to mealworm-based pet food

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The acceptance of insects in the diet of companion animals has received considerable attention in recent years. The abstract presents the results of a survey conducted in veterinary clinics to assess public perception of the use of mealworm-based food in the diet of dogs. The survey aimed to determine the level of acceptance, market opportunities and factors influencing the willingness of the public to include insect-based food in the diet of their pets. The study involved 436 dog owners. Insect-based pet food was given to 55 dogs and assessed for acceptability and willingness to re-consume at a follow-up clinic visit.

Approximately 65% of respondents were open to the idea of introducing insect-based pet food to the diet of companion animals. This percentage increased significantly to about 85% when insect-based food was associated with added values, such as hypoallergenic or health-promoting effects supported by reliable clinical studies. Interestingly, the survey revealed a significant propensity for dog owners whose pets suffered from food allergies to eat an insect-based diet. Of the dogs, 52 (94.45%) individuals consumed the pet food and re-consumed it at the next visit.

This study highlights a market opportunity for a mealworm-based pet food in the veterinary industry, provided it is rigorously tested and offers added value backed by credible research. In addition, investing in research to determine the health and nutritional benefits of insect-based foods is critical to increasing perceived value among pet owners.

By meeting rigorous safety standards, mealworm-based pet foods can capitalize on the market opportunity presented by the growing acceptance of edible insects in animal diets. The research highlights the importance of safety testing and scientific evidence in gaining consumer trust and establishing a strong position in the insect-based pet food market.

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CIPROMED: Alternative proteins exploitation in the Mediterranean food and feed chains

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Mediterranean food and feed value chains largely depend on protein imports to meet the increasing human nutritional needs, as well as the needs of the aquaculture and the livestock sector. As a result, Mediterranean food and feed production chains are prone to interruptions of the supply chain due to unexpected events and circumstances. To address this problem, Mediterranean countries seek for alternative protein sources that can be locally and sustainably produced. CIPROMED (Circular and Inclusive utilisation of alternative PROteins in the MEDiterranean value chains) is a recently launched research project that aspires to fully characterize and evaluate protein fractions from alternative raw materials, i.e., insects, microalgae and legumes, that will be extracted through innovative green extraction technologies. These will be further exploited as ingredients of innovative food and feed products. Furthermore, CIPROMED aims to upcycle and valorise agri-industrial side-streams for insect rearing and microalgae cultivation. Finally, the environmental impact and economic implications of the new products and technologies will be assessed using LCA methodologies. This research is supported by the EU-PRIMA program project CIPROMED.

Management models to promote sustainability and resilience of agricultural production systems – The AGRITECH project

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The AGRITECH project aims at using emerging technologies for the sustainable development of agri-food production, with the aim of promoting adaptation to climate change, reducing the environmental impact in the agri-food system, and developing marginal areas, safety, traceability and typicality of the supply chains. AGRITECH promotes the development of models for the sustainability and resilience of agricultural systems, focusing on circular management for the exploitation of waste materials. Indeed, meeting an ever-increasing demand for the food and feed, as well as managing organic waste, has become a major global challenge. Organic waste (OW) leads to significant environmental (i.e., greenhouse gas emissions, landfill disposal) and economic footprints (cost of disposal). The black soldier fly larvae (BSFL) (*Hermetia illucens*) can efficiently bioconvert OW of multiple origin (reducing them by up to 65%) into valuable nutrients (proteins, lipids) that can find uses in animal feed. Mixing OW having different nutritive values enables to optimise the bioconversion process, and to formulate specific diets for larvae able to meet their nutritional requirements. The bioconversion process leads to the production of insect-derived products (meal, oil) that can replace expensive feed ingredients normally used for livestock (e.g., fishmeal, soybean meal), mainly for fish, poultry and pig. In 2022, the feed market for these animals overcome 900 million tons, and there is a global struggling for high-quality proteins and other valuable nutrients. Beyond the nutrients supply, insects are also a source of active bio-compounds (chitin, lauric acid, antimicrobial peptides) that are able to exert a positive impact on animal health and act as potential antibiotic replacers. Research on these topics have mostly been performed on small-scale only, while the validation on field is still lacking. In the frame of the AGRITECH project, OW are upcycled using BSFL to produce meal and fat to be used in fish and pig large scale trials. The AGRITECH National Research Center project received funding from the European Union Next-GenerationEU (Piano Nazionale di Ripresa e Resilienza (PNRR).

Individual reproduction performance as an efficient method of testing larval treatments on reproduction in adult stage

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Research on breeding optimization is essential for insect rearing companies, since they often have to self-supply their production of larvae. Therefore, companies must have an efficient method of testing effects of treatments on the reproduction performance of their insects. In industrial settings, the black soldier fly breed in group and mating is often done in cages. For research questions on a population level, e.g. mating rate or insect density, experiments in cages are essential. Unfortunately, when testing for effects on an individual level, cage based experimental designs introduce too many confounding effects, such as fly density, sex ratio and mating rate. Furthermore, to ensure statistical power, experiments in cages can require a large number of flies and cages, which may not be operationally feasible for large scale tests or smaller R&D teams. Here, we propose an experimental design in which post-mating females are isolated to obtain their individual clutch weight rather than an overall oviposition for the group. For logistical reasons, larvae are reared together, and flies emerge in groups, with several females from each group subsequently isolated. However, this experiment design creates a hierarchical data structure and a mixed model analysis, where the rearing bins is a random effect, is needed. Furthermore, we suggest having several rearing bins by treatment to ensure sufficient replication. This method offers an efficient way of testing the effects of treatments on reproduction specifically those induced at the preadult stage, such as larval diet on individual reproduction.

Microbiological quality of edible insect products

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When assessing the microbiological aspects of edible insects, it is necessary to consider that insects are natural carriers of microorganisms and may harbor a complex autochthonous microbial population. Therefore, it is essential to investigate the microbiota of edible insects to evaluate potential risks to human health.

A total of 20 samples from various categories of products containing *Acheta domesticus* in whole or processed form into cricket powder were selected for microbiological analysis. In addition to bars and pasta, testing was conducted on five other categories of products to detect the presence of nine groups of microorganisms most found in insects. All tests were conducted using cultivation methods following standard procedures and applicable ISO standards, with the subsequent identification of selected parameters using MALDI-TOF mass spectrometry.

Upon comparing the obtained results with data from scientific studies, it can be concluded that thermal processing helped eliminate some species of microorganisms. However, despite technological processing, a high number of mesophilic aerobic microorganisms remained, primarily. It is also worth noting that while insects are one source of microbiological issues, other ingredients used in the food, especially spices, are often a source of undesirable microorganisms.

Effect of larval weight on quantity and quality of mealworm protein isolates

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Larvae of the flour beetle (*Tenebrio molitor*) go through a larval stage in which they achieve substantial growth and typically reach a weight of 80-140 mg before pupation, depending on the substrate and other rearing conditions. Commercially traded mealworms are not necessarily standardized such that weight variations between batches may occur.

The aim of this study was to determine how such variations in the larval weight affect the protein yield and techno-functional properties of protein isolates. To determine whether possible differences between small and large larvae could be additionally influenced by the extraction procedure, proteins from oven dried small (70 mg) and large (105 mg) mealworms were isolated by alkaline extraction and acidic extraction plus salting out. The protein isolates obtained were quantified and analyzed in terms of their composition and for selected functional properties.

Extraction efficiencies related to the protein content of the starting material ranged between 7,9 % and 32,3 % and decreased in the order large/alkaline > small/alkaline >> small/ acidic > large/acidic. Techno-functional properties varied only marginally between small and large larvae. By contrast, significant differences were found between the two extraction methods. The alkaline extracts contained larger proteins and showed potential as emulsifiers, whereas the acidic extracts contained a large fraction of smaller proteins and exhibited both emulsifying and gel-forming properties. Furthermore, the isoelectric point of the alkaline extracts was at substantially lower pH than that of the acidic extracts.

With regard to protein yield, larger larvae are preferable. Irrespective of the extraction method, certain techno-functional properties vary to a small extent depending on the larval weight.

Enzymatic conversion of black soldier fly larvae oil to diols for the preparation of biogenic polyurethane foams

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Biogenic polyurethanes are one possibility to decrease the strain on fossil resources and improve the sustainability of construction materials by harnessing agricultural sidestreams for the generation of foam insulation. Black Soldier Fly Larvae (BSFL) convert agricultural waste to feedstock, which can be used for chemical synthesis of materials, such as surfactants, lubricants, or polymeric foams.
Here, the oil of the BSFL was transesterified enzymatically to monoglycerides, eliminating the need for high temperatures and the use of strong alkali. Different feed substrates were used for larvae cultivation, resulting in specific fatty acid patterns. After extraction and purification, the oil was converted in a continuous process using immobilized Candida antarctica lipase B (CALB) in a packed bed reactor at 50°C for 4 h. The reaction progress was monitored with HPLC during the reaction time. Samples were drawn every 10 minutes and analyzed. The results show significant conversion to monoglycerides after 170 minutes. The reactions were also conducted with tricaprylin and plant oils as reference. The conversions of all substrates were completed after a maximum of 4 h reaction time.
The mixtures obtained were used to prepare biogenic polyurethane foams by reaction with 4,4'-MDI and without further purification. The resulting foams were characterized for their mechanical and thermal properties as well as microscopically to characterize pore sizes and pore size distribution. The results show that enzymatic conversion of insect derived fats is an effective and energy efficient process with a low carbon footprint to produce feedstock for biogenic polyurethane foams, suitable as insulating materials. All process steps involved can be transferred to industrial scale.

Simultaneous determination of chitin and amino acids by Ion-exchange-chromatography

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Main arguments for a more extensive utilization of insects in food and feed are based on protein content and amino acid composition. The common method for the determination of the protein content is the Kjeldahl´s method, in which the nitrogen content of a sample is multiplied with a conversion factor (often F=6.25) to calculate the amount of raw protein. This is suitable for matrices that contain only very small amounts of nitrogen containing ingredients, other than proteins. In insects, chitin as polymer of N-acetylglucosamine units leads to an overestimation of the protein content. Thus, the determination of chitin is important for a correct evaluation of the nutritional value of insects.

Under acidic conditions, chitin is hydrolysed to glucosamine monomers, which could be separated by cation-exchange-chromatography and detected with ninhydrin, a classical method for amino acid analyses. We therefore investigated the possibility of a simultaneous analysis of chitin/ glucosamine and amino acid composition after acidic hydrolysis of the material.

Within the chromatogram obtained for amino acid analysis, glucosamine eluted between tyrosine and phenylalanine in amino acid/ glucosamine mixtures as well as in hydrolyzed mealworm flour. A linear regression was established from 0.3 to 20 nmol per injection. Recovery rates were between 60 and 115% in spiked mixtures. Further optimization and validation procedures concerning sample preparation and chromatography are in progress.

We therefore state that ion-exchange-chromatography with post-column-derivatisation with ninhydrin is a promising tool for a simultaneous determination of amino acids and chitin/ glucosamine.

Insect-based bioconversion of agricultural by-products into sustainable food

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About one third of all food produced worldwide is lost or wasted along the food value chain. New strategies are necessary to use our limited resources e.g. water, energy and arable land for sustainable food production more efficiently. Insects are able to convert organic substrates into protein biomass more efficiently than conventional livestock. Our objective is therefore to produce valuable protein biomass for food applications by bio-converting agricultural by-products using edible insects.
The first step involves processing the insects into protein-rich ingredients. Since insect biomass contains significant amounts of fat and chitin, different extraction and fractionation processes are established. Processing steps include drying, milling and deoiling to derive full fat and defatted insect flours. The insect flours are further fractionated by dry and wet methods to produce protein concentrates and isolates. Subsequently, the functional properties of the insect concentrates and isolates, such as gelling behaviour, emulsifying capacity and water binding capacity, are determined and compared to commercially available plant protein concentrates and isolates. Other components such as lipids and chitin that are derived after fractionation are characterized regarding their composition and relevant properties to evaluate their anticipated market potential. To test the in-sect concentrates and isolates for their applicability in food products, they are texturized by extrusion processing and tested in snack products or meat alternatives, such as buger patties.

The bioaccumulation of rare earth elements in *Tenebrio molitor*

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In recent years, many scientific publications have focused on phenomenon of the bioaccumulation of heavy metals by various types of insects. However, the insect *Tenebrio molitor* has not yet been tested for bioaccumulation of rare earth elements (REE). This ability may be valuable in entomoremediation of contaminated or nuisance waste biomass. Municipal sewage sludge is one example of a waste that could potentially contain high amounts of REE. It is estimated that the annual global production of sewage sludge reaches about 359.4×10^9 m^3, and its disposal and management methods do not take into account elemental treatment and recovery. The purpose of the study was to determine the potential for bioaccumulation of REEs by *T. molitor*.

The experiment consisted of breeding yellow mealworm (*T. molitor* larvae) on digested municipal sewage sludge obtained from the “Hajdów” wastewater treatment plant of Lublin and near cities. The second variant included the same sludge with the addition of EDTA as a metal complexing agent in order to test whether its presence would increase the uptake of metals by insects. The experiment lasted for 55 days, until the larvae were no longer observed to pupate.

Elemental content analysis was conducted using inductively coupled plasma atomic emission spectroscopy (ICP-AES) in insect samples from three developmental stages of *T. molitor*: larvae, pupae and adult beetles. Bioaccumulation of REEs such as dysprosium (Dy) and lutetium (Lu) was demonstrated.

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Performance of selectively bred black soldier fly larvae in a production facility

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Selective breeding to genetically improve Black Soldier Flies (BSF) (*Hermetia illucens*) is integral to enabling BSF producers to scale faster, enhance productivity and increase their profit margin. The breeding objectives of Beta Bugs Limited's selective breeding programme (BP) are driven by the commercial requirements of key agribusiness markets for BSF products. The continually improved product- HiPer-Fly (HPF) ® - is the outcome of improvement of production traits (larval mass, egg clutch mass, development time) and fitness traits (survivability) targeting juvenile and adult life stages within the BP.

The BP goal is to achieve genetic improvements which are permanent regardless of the environment, such as feed stock, which can fluctuate depending on the variable rearing conditions provided in customer's facilities. The performance of HPF larval genetics at an industrial scale was evaluated by comparison with Beta Bugs Ltd's unselected product Just-Fly (JF) ™ at the company's production site. Replicate groups of HPF and JF larvae were reared at 28 °C and 70 % relative humidity to 5 days old (DOLs) on a high nutrient starter diet (meal 1 (M1)). Larvae were then extracted and sampled to obtain an initial population count by dividing the total larval mass by the average individual larval mass. The 5 DOLs were then transferred to substrate comprised of spent brewer's grains supplemented with chicken feed (meal 2 (M2)) to complete growth and development until harvest stage. Post-extraction population numbers were estimated, and development time recorded as days between M2 and extraction.

The performance of HPF larvae exceeded that of the unimproved line for key traits, notably with regard to total larval biomass yield and individual larval mass (mg). The results of the benchmarking trial indicate that following transfer of HPF into a mass production facility, subjected to the same environment and husbandry as an unimproved BSF line, and no longer subject to active precision breeding for multiple generations, HPF continues to perform by retaining key genetic improvements.

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

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

Influence of different substrates based on by-products of food processing on the performance of black soldier fly larvae

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The larvae of black soldier fly (BSFL) (*Hermetia illucens*) represent a protein-rich feed alternative to soybean meal for livestock nutrition. However, BSFL are commonly reared using high amounts of milling by-products that could be directly utilized in livestock nutrition. Thus, there is a need for exploring alternative feed substrates for BSFL.

In an experiment, eleven feed substrates were tested, including a control diet (CD) comprising 14.7% wheat bran (WB), 8.6% wheat semolina bran (WSB), 0.44% trace mineral - vitamin premix (pig), 0.66% feed acid, and 75.6% water. In the experimental diets, WB and WSB were quantitatively replaced by 15, 30, or 50%, of grape pomace (GP), elder pomace (EP), or potato steam tray (PST). Additionally, two diets were prepared replacing 50% of WB and WSB by a mixture of 25% GP and 25% PST, or a mixture of 25% EP and 25% PST. The substitution was based on dry matter (DM), maintaining a constant DM of 22% for each diet. The trial was conducted with six boxes per substrate with one box forming one replicate. Each replication contained approximately 11,000 larvae, 10 kg of the respective diet and was placed in a climate chamber (temperature of 33°C, relative air humidity: 70 %). After 7 days of feeding, the BSFL were sieved, weighed and the total weight and the feed conversion ratio (FCR) were statistically analyzed by one-way ANOVA with the software package SAS. Larvae fed with CD showed a total weight of 1,711 g/box. Similar weights were observed feeding substrates including 15% GP, up to 30% PST, or a mixture of GP/PST ($p>0.05$). Reduced weights were observed for BSFL fed substrates with 50% GP or 50% PST ($p<0.05$; 1.058 g and 1.130 g, respectively). The FCR for CD was 1.29 kg/kg and does not differ from larvae feeding substrates including 15% GP, up to 30% PST, or the GP/PST mixture ($p>0.05$). The FCR was highest when feeding 50% GP (2.10 kg/kg) and 50% PST (2.05 kg/kg) ($p<0.05$).

In conclusion, GP (up to 15%), PST (up to 30%), and a mixture of GP/PST (25/25%) could be well utilized to reduce the proportion of high-quality mill by-products in substrates for BSFL.

Novel approaches to age determination of *Hermetia illucens* adult flies (Diptera: Stratiomyidae)

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The Black Soldier Fly (BSF) plays a key role in producing protein, oil and fertilizer for feed and agriculture industries. One of the most critical factors in rearing *H. illucens* is the ability to determine the chronological age of adult flies. Little information is known about age determination in BSF. Recent studies present fragmented data and require clarifications and additions. Studying age criteria will allow breeders to organize a more effective mating process of *H. illucens* and increase their overall productivity.

The goal of our research was to evaluate novel approaches to estimating the age of adult BSF.

The following tasks were performed during this research: (1) to investigate the transparent 'windows' of the first abdominal segment as age indicator in BSF; (2) to estimate the age of *H. illucens* females using changes in the reproductive system during egg formation; (3) to analyze the cuticular bands as age criteria in BSF.

The adult flies were kept at constant temperature and humidity. In all cases, samples of *H. illucens* were taken at 24 hourly intervals for 10 days. Flies for dissection were killed in alcohol and dissected immediately. The total number of flies investigated is 960. All obtained quantitative data was processed using the IBM SPSS Statistics software. We found a statistically significant difference in 'window' fullness according to the age in males ($F=32,3$, $p<0,001$) and females ($F=26,2$, $p<0,001$). Furthermore, sharp decrease in female`s 'window' fullness on the 7^{th} day after emergence has been observed. The decrease in the fullness of the abdominal 'window' was more gradual in males. The ovarian development passes through four stages outlined in H. Watabe and K. Beppu (1977) for *Drosophila sordidula* and *D. testacea*. The chronological age that corresponds to a particular change in the female reproductive system has been described. We found no differences in the ovarian development of 7-day-old mated females and older. Daily cuticular banding on the mesothoracic post-phragmata in BSF was also observed. Growth layers are clearly visible in both males and females and up to 8 cuticular bands can be counted. The phragmata in *H. illucens* reaches maximum size 8 days after emergence.

These results show that the studied approaches can be successfully used during the breeding process for precise determination of adult BSF chronological age.

Optimization of black soldier fly larvae (BSFL) feed conversion on industrial high-fiber side streams

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Population growth and increasing demand for natural resources contributed to waste production from the industry. A different approach is essential for providing sustainable food and feed to support population growth. Insect farming is a novel and rapidly growing sector with great potential for the circular bioeconomy. Insects have received more attention as they provide an alternative protein source for sustainable feed production. In addition, insects have proven to be able to upcycle industrial side streams into valued biomass which contains proteins, lipids, and chitin.

Industrial side streams such as potato pulp (starch manufacturing byproduct) and apple pomace (byproduct from the beverage industry) contain high amounts of fiber and have a low protein and lipid content. This poses a challenge when used as feed for various animals. Our results show that the larvae of the black soldier fly (*Hermetia illucens*) can be reared on both, potato pulp and apple pomace. Although the developmental time was much longer and the final larval weight was lower compared to the control group reared on chicken feed, the other developmental parameters of larvae and flies were almost similar to the control group. Therefore, we conclude that *H. illucens* is able to adapt to high-fiber diets and can develop with low amounts of lipids and protein in the respective side streams.

We also analyzed the gut microbiome using amplicon sequencing to observe the adaptation to the high-fiber side streams. Furthermore, we have isolated various bacterial and fungal strains with cellulolytic activity from larvae reared on high-fiber diets, which might have the potential as probiotics to optimize feed conversion.

Rearing black soldier fly larvae (BSFL) on industrial side-streams/by-products gaining valued frass as fertilizer

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The larvae of the black soldier fly (*Hermetia illucens*), are a source of proteins and lipids and are already used as feed for poultry and in aquaculture. By using by-products from local industrial partner as a feed resource for BSFL, valuable biomass with a high protein and lipid content can be generated in an economically viable way. This could avoid complex and costly composting or thermal recycling. The residues from BSFL rearing, so-called frass, contains leftover feed as well as insect droppings and can be used as a valuable fertilizer. Its composition is influenced by the larvae's metabolism and the supplied feed composition.

In this study we used potato peelings, potato pulp, apple pomace, and rapeseed press cake as feed for BSFL rearing and analyzed the frass obtained after larval development on the respective feed. Our study demonstrates that the frass' chemical composition varies depending on the feed, including differences in the nitrogen, phosphorus, and potassium (NPK) composition. We could also show, that the EU legal requirement of hygienizing the frass at 70°C not only reduces potentially harmful coliform bacteria (including *E. coli*) but also the total viable count, thus probably including bacteria responsible for soil improvement, such as nitrogen fixation, phytohormone production, and organic acid production. In addition, we used cultivation-dependent and cultivation-independent (amplicon sequencing) methods to analyze microbial composition and to identify strains with known plant growth-promoting properties.

PauseM-Direct - Nursery in a package

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The use of Black Soldier Fly larvae in the waste management and animal feed industries has highly increased in recent years. Consequently, the use of larvae at dispersed and local facilities, rather than large insect factories, is starting to develop. At FreezeM, we developed PauseM, a technology suspending the growth of freshly hatched BSF larvae for 14 days. Neonates are accurately counted, dosed into packages, and shipped globally to various rearing facilities, replacing the need for self-breeding. Once unpacked on nursery feed at the facility, the neonates restart growing at a faster rate compared to freshly hatched neonates. In facilities that lack infrastructure or expertise to maintain a nursery, there is a need of an alternative method for activating the BSF larvae. For this purpose, we developed PauseM-Direct, which enables the nursery phase to be done in the package and neonates to be transferred directly to the rearing phase. PauseM-Direct packages were supplemented with feed and incubated in a rearing room for several days. Then, neonates were transferred directly to rearing crates containing rearing feed, which resulted in high larvae weight, while sustaining high survival rates. Remarkably, the rearing period of larvae from PauseM- Direct packages was significantly shorter than from the regular PauseM packages utilized directly on rearing trays. Taken together, our results suggest that the use of PauseM-Direct prepares the neonates in the package for their transfer to rearing, probably by initiating a metabolic change, making the nursery stage unnecessary. In conclusion, our newly developed product and protocol offers an even more simplified and rapid way to rear larvae at local and small BSF rearing facilities.

Assessing the taxonomic and functional diversity of the black soldier fly microbiome

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To understand the importance of microorganisms in development and performance of industrially produced insects, such as larvae of the Black Soldier Fly (BSF, *Hermetia illucens*), fundamental knowledge of the taxonomy and functions of the microbiome is essential. Currently, the most commonly used method in microbiome research is sequencing of taxonomic markers such as the ribosomal RNA (rRNA) genes (also known as metagenetics or metataxonomics). Although amplicon sequencing is fast and cost-efficient, the taxonomic resolution is often insufficient to reliably differentiate microorganisms at species and/or strain level. In contrast, although more expensive and analytically more demanding, shotgun metagenomic sequencing provides not only better taxonomic resolution, it also allows simultaneous identification of both eukaryotic and prokaryotic species and provides relevant functional information. In this study, both 16S rRNA gene amplicon and shotgun metagenomic sequencing were compared to profile the microbiome of the gut and whole bodies of BSF larvae. We specifically focused on resolving the impact of different rearing conditions on the taxonomic and functional diversity of the microbiome of BSF larvae.

Effect of additives to blanching water of mealworms

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Insects are increasingly used in the food and feed industry. The most commonly used first step in processing insects is blanching them as it reduces the microbial load and enzymatic browning reactions. For process optimization, it was investigated whether adding additives to the blanching water reduces leaching of water-soluble nutrients. In this experiment we focussed on the effect of the additives in blanching water on leaching behaviour of mealworms (*Tenebrio molitor*), tested on industrial scale. A test was conducted with 0.1% $NaHCO_3$ which should reduce leaching. In addition, a test using 0.1% citric acid as antioxidant was performed. The blanched mealworms were analysed and compared to a control group of mealworms which were blanched without additives.

Mealworms were blanched for 2 minutes in boiling water, oven-dried at 60°C and grinded for nutritional analysis. Proximate analysis (dry matter, ash, protein, fat, carbohydrates), mineral content (P, Mg, K, Na, Ca, Zn, Cu, Fe, Mn) and vitamin B12 and E were determined in the different mealworm samples.

No significant differences in the proximate composition were found between the control group and the mealworms blanched with additive. This applies for both additives. No significant differences were observed in mineral concentrations and vitamin B12 content. Mealworms blanched with citric acid have statistically less vitamin E in their oil fraction compared to the control. However, when recalculated to the vitamin E content in fresh samples, the difference is no longer significant.

In order to evaluate the leaching process in more detail, future experiments should include chemical analysis of the water before and after blanching.

Environmental impacts of *Hermetia illucens* protein production: A life cycle assessment and comparative analysis

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Environmental impacts resulting from food production are significant contributors to climate change and overall sustainability challenges. With the aquaculture industry experiencing rapid growth, the global demand for sustainable protein sources has led to increased interest in alternative feed ingredients for aquaculture. Insect meal production is generally considered more environmentally friendly than traditional ingredients, but further research is needed to fully understand its environmental performance. This study evaluated the environmental impacts of producing *Hermetia illucens* protein using a life cycle assessment (LCA) approach. A hotspot analysis and a comparative analysis were performed to assess the environmental performance of *H. illucens* protein compared to other traditional protein sources used in aquaculture.

Land use, energy use, freshwater ecotoxicity, climate change, and water use were identified as the most relevant impact categories. Raw material production and insect meal production stages accounted for over 80% of the environmental impacts. Specifically, the cultivation, transport, drying, and storage of corn and wheat bran used as a substrate for larval feeding significantly contributed to the environmental impacts during raw material production in all selected impact categories. The production of the electricity consumed in the insect meal production stage was identified as the most relevant process in land use, fossil resource depletion, freshwater ecotoxicity, and climate change. Overall, protein from *H. illucens* presented less favorable results in terms of environmental impacts compared to fishmeal and soybean meal protein. As a potential solution, an additional LCA considered using agri-food waste as insect feed and renewable energy sources was developed. These changes significantly reduced environmental impacts in all selected impact categories and favourable environmental results were obtained in comparison to fishmeal and soybean meal. This study emphasizes the need to optimize *H. illucens* process production in order to improve its sustainability in comparison with traditional alternatives.

Novel biomolecules from native myrmicine ants exploited for new applications

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Many of the more than 14,000 extant ant species evolved a remarkable chemical defense system in the form of venom. Venoms are a complex blend of potent polypeptides. Despite their promising translational potential unveiled by recent works, most ant venoms have not been examined yet and a plethora of potentially valuable biomolecules remain undiscovered. The venom of the smaller Central European representatives of the Myrmicinae in particular have so far remained virtually unexplored.

Here, we screened a previously generated venom gland transcriptome from *Myrmica rubra* and *Myrmica ruginodis* using known antimicrobial peptides (AMPs) as queries and identified ten novel transcripts encoding AMP-like toxins. All ten mature peptides were synthesized and subjected to functional profiling. This included tests for cytotoxicity as well as insecticidal, antiviral and antimicrobial effects. In particular, bioactivity testing against a panel of seven bacterial strains revealed a broad spectrum of activity, including potent antipathogenic activity of the toxin U-MYRTX-Mrug5a. This effect was accompanied by low cytotoxicity rendering it a promising lead structure for further development.

Self-selection feed design for crickets (*Gryllus Bimaculatus*) at different growth stages using food waste

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As the world's population grows, food and feed security is becoming an important issue; in 2013, the Food and Agriculture Organization of the United Nations (FAO) announced that insects could be one of the solutions to future food shortages, and insect proteins have since gained attention. Omnivorous insects have attracted attention because of their potential to utilize food waste as feed during production. However, the use of food waste as feed has not progressed since food waste generated in different regions differs and the design of insect feed has not been established. Therefore, in this study, with the utilization of food waste in Japan in mind, we designed a feed for *Gryllus bimaculatus* using self-selection, which is an instinct of the organism, and obtained data at each growth stage and designed the feed from the obtained data. The results showed that when self-selected diets were designed considering Japanese food waste, it was possible to achieve growth levels comparable to those used in the industry (cricket weight results at 4 weeks post-hatching with an initial input of 1 commercial diet, mean standard ± deviation : 208.8 ± 45.7 g, self-selection diet design: 232.5 ± 26.2 g). The results of this indicate that data on self-selection by growth stage may be used more efficiently when designing diets for cricket production. Future feed design will examine changes in productivity, egg laying and hatching, and allergies when insects are used as human food.

The potential of monitoring system for *Gryllus bimaculatus* based on loadcells and computer vision

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Crickets, *Gryllus bimaculatus*, have gained recognition as a promising alternative source of protein. Due to their short interval of production time, offer a time-saving advantage in comparison to traditional livestock. Furthermore, cricket production is associated with a lower carbon footprint. While production has been made in this field, there are several aspects of cricket biology and behavior that require further investigation and comprehension. Several farms have begun integrating sensors, humidity and temperature, into their cricket rearing facilities. These sensors serve as monitoring tools that provide valuable insights into the cricket farm environment. However, the relationship between these measurements and the behaviors of crickets has not been extensively explored. This paper explores the potential of using various sensors to monitor cricket behavior with the aim of developing monitoring systems for both research and production purposes. During the data collection period using sensors, various parameters such as the weight of different zones, temperature, and humidity data are recorded and stored in a database. A server is employed to run a Python program specifically designed for processing streaming video obtained from an IP camera. The program utilizes YOLOv5 to detect within each zone of video and store the number of detected crickets. Additionally, a dashboard has been developed to visualize and demonstrate the key parameters. The result show, it has potential to be used for various kind of utilizing such as training model to estimate the number cricket using weight data, diagnosing the abnormal pattern of consuming rate, estimating the feed run-out time, moreover, this approach can be utilized for feed design research, expected to find effective composition and can take allergies into account, which involves periodically measuring the diet weight. In conclusion, the developed system offers the capability to continuously measure and monitor crucial parameters. Its potential applications extend to both research and production systems in the future.

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

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The consumer acceptance of insects as food can be increased by processing, for example by isolating the proteins as functional ingredients. Therefore, the aim of our work was an extraction of mealworm (*T. molitor*) proteins following the example of Osborne fractionation with subsequent evaluation of the biological value of the protein isolates. A fractionation method for soldier fly larvae (*H. illucens*), yielding four protein isolates based on their solubility, was optimized for application to mealworms. Amino acid composition was determined using three different methods. For tryptophan, hydrolysis was performed under alkaline conditions with barium hydroxide followed by quantification using RP-HPLC-UV and internal standard N-α-methyltryptophan. The sulfur-containing amino acids cysteine and methionine were determined by amino acid analysis (ASA) after oxidation followed by hydrochloric acid hydrolysis using cation exchange chromatography and ninhydrin post-column derivatization. The remaining proteinogenic amino acids were quantified after hydrochloric acid hydrolysis followed by ASA. The protein contents of the fractions and the protein yields were calculated from the sum of the total amino acids. For classification of biological value, the sum of indispensable amino acids (IAAs) was determined, and limiting amino acids with associated amino acid scores (AAS) were calculated in comparison to WHO recommendations.

The most yielding fractions were the alkaline-derived fraction with 38%, and the aqueous fraction with 19%. Only 11% of the proteins remained in the insoluble residue.

The biological value was assessed for the defatted insect meal, as well as for the two fractions with the highest yield. The meal showed the best values with 572 mg IAAs/g protein and an AAS of 1.21 (methionine). The aqueous extracted fraction follows with 568 mg IAAs/mg protein and an AAS of 1.18 (methionine). With an AAS of 0.82, limited by cysteine, the alkali-derived fraction has the lowest biological value.

Thus, mealworms and their protein isolates were shown to have a high nutritional quality comparable to conventional animal proteins, which is limited by sulfur-containing amino acids. Osborne fractionation is a suitable tool to obtain defined protein isolates for further applications.

The use of waste polystyrene as feed for mealworms (*Tenebrio molitor*)

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This study aimed to determine what percentage of the feed provided to the mealworm larvae of *Tenebrio molitor* (MWL) could be substituted by polystyrene (PS) whilst retaining mealworm viability. The survival and growth rates, as well as the general performance of the larvae, were followed for a 6-week duration.

Four test groups in three independent replicates of MWL, each with 300 larvae at the 8th instar, were fed on varying PS-bran percentages for six weeks under standard growth environment conditions. The four feeding regimes were 0, 50, 75 and 100% PS. The remainder of the feed for the 0, 50 and 75% groups was wheat bran. A 5g carrot supplement was provided for all groups weekly. The mealworms were monitored for mortality, percentage weight gain (%WG) and percentage length gain (%LG), and changes in their lipid and protein content at the end of the 6-week trial.

Feeding solely PS did not provide the best degradation of PS, with only 27.39% degradation in six weeks. The 75% PS treatment had the lowest %PS consumption at 21.74%. The best alternative was to provide a feed consisting of 50-50 PS-wheat bran supplemented with carrots giving the highest %PS consumption at 31.06%. Furthermore, over six weeks, the 50% PS treatment larvae had the best %LG and %WG at 47.1% and 278.6%, respectively. By comparison, the 100% PS treatment achieved a %LG and %WG of 36.6% and 205.4 respectively. Mortalities of the 50% PS treatment were of 54.7%, similar to the 75% PS group, whilst, for the 100% PS, mortalities were 73.89%. The control group achieved the lowest mortalities at 22.78%.

An additional test was conducted to investigate if a diet consisting of 50% PS significantly affected the protein and lipid content of the MWL compared to the control group. Analysis showed a statistically significant difference in protein content, with the 50% PS diet resulting in a higher protein content of 56.49% and 25.51 % lipid content, on a dry matter basis, when compared to the control group.

In conclusion, the 50% PS treatment larvae gave the best larval growth and survival rates out of the three treatments with PS provided in their diet, whilst the 100% PS gave poor results as PS degradation was not as efficient in this test group. A better approach to making use of waste PS is actually to provide the PS as part of a diet supplemented with natural feeds, such as wheat bran and carrots. For the commercialising of this concept, further studies need to be conducted.

Edible insects in arresting inflammatory diseases and food insecurity

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Several communities around the world, have been traditionally eating insects since time immemorial. Food insecurity emerging from climate change, extreme weather events, ongoing global supply chain problems, and unpredictable geopolitical conflicts will impact all nations especially the growing populations of low- and middle-income countries. In pursuance of more accessible, affordable, and sustainable nutrition, insects have come under the limelight and increased scrutiny in recent times due to their perceived importance as a part of multifaceted strategies for achieving global food security.

This study consists of two parts: a survey of entomophagy attitudes and evaluation of anti-inflammatory activities of several edible insect species identified from the survey. Additionally, vitamin, and macronutrient content of these species were also investigated. It was found that 25 unique species of edible insects were consumed in north-east India which falls within the Indo-Burma Biodiversity hotspot. This study also found that besides being highly proteinaceous, these edible insects possessed peptides with high anti-inflammatory activity. Inflammation in the body has been found to be the causative agent for most chronic diseases. Inflammation also hastens the ageing process. The edible insects in this study, with their observed nutrient profile and anti-inflammatory properties, are a suitable addition or replacement to diets across the globe. They are also fitted be a solution to food insecurity in the low- and middle-income nations and the ever-increasing demand for food in general. An interesting finding of this investigation was that the major reason for consumption of insects among the studied communities was their superior taste. Nutritional benefits are recent revelations and is often not a factor for consumption among the traditional insect-consuming communities. This finding can be instrumental in overcoming the 'yuck factor' which is often associated with edible insects among the non-insect eating population of the globe. The present study also highlights the areas in edible insect literature which need more research.

Black soldier fly larvae production performances are optimized by the supplementation of a multi-carbohydrase

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Insects are a promising way to upcycle food and feed waste. They are also a promising source of proteins and fats for aquaculture, pet and poultry feed, and can partially replace vegetal proteins, thus decreasing the carbon footprint of meat production. Still, the development of insect meal production must be improved to have efficient and economical ways to produce insect larvae. In this study, black soldier fly (*Hermetia Illucens*) raised on food waste were tested for their efficiency on performance and cost, when a multi-carbohydrase additive (containing xylanase (12,500 U/ml), glucanase (8,600U/ml) and arabinofuranosidase) was added or not in the larvae feed. A 7-day trial starting with 1st instar black soldier fly larvae (BSFL) was run with 4 dietary treatments x 6 crate replicates: control, control plus a multi-carbohydrase dose of 100, 200 or 400 ml/ton larvae feed. The average initial weight was 2.41 mg/larvae, and those larvae were equally inoculated in 12 kg of feed commonly used in a production system. The additive was added during the fermentation of feed for 3 days prior to the trial. Results: the treatments with multi-carbohydrase at 100 and 200 ml/ton presented 8.5% and 12.7% significantly ($p<0.05$) better fresh larvae biomass production than control on the whole period, respectively. Considering larvae biomass and feed residue on the whole period, the feed conversion of these treatments was statistically different: 3.49^{a}; 2.55^{b}; 2.26^{c}; 2.15^{c}; respectively. The fresh larvae presented just numerical increase in crude fat content for all treatments when compared to control: 27.6%; 33.0%; 34.1%; 37.0%, respectively, and no differences on the crude protein content: 52.7%; 47.7%; 47.8%; 46.7%. Besides that, the cost of insect meal per treatment demonstrated to be lower by 32% when the additive was added in the larvae feed. Conclusion: the use of multi-carbohydrases brings high benefits in terms of performance and cost to produce BSFL by the better feed conversion and fat production, improving the food recycling. Further studies are necessary to confirm those effects and extend this use under different conditions of production.

Black soldier fly larvae production performances are optimized by the supplementation of HMTBa

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Insects are a promising way to upcycle food and feed waste. They are also a promising source of proteins and fats for aquaculture, pet and poultry feed, and can partially replace vegetal proteins, thus decreasing the carbon footprint of meat production. Still, the development of insect meal production has to be improved to have efficient and economical ways to produce insect larvae. In this study, black soldier fly (*Hermetia Illucens*) raised on food waste were tested for their efficiency on performance and cost, when a source of methionine: Hydroxy-methylthiobutanoic acid (HMTBa) was added or not in the larvae feed. A 7-day trial starting with 1st instar black soldier fly larvae (BSFL) was run with 4 dietary treatments x 6 crate replicates: control, control plus HMTBa at doses 0.08%, 0.16% or 0.32%. The average initial weight was 2.41 mg/larvae, and the larvae were equally inoculated in 12 kg of feed commonly used in a production system. The HMTBa additive was added during the fermentation of feed for 3 days prior to the trial. Results: HMTBa used at 0.16% and 0.32% presented 11.9% and 10.4% significatively ($p<0.05\%$) higher fresh larvae biomass production than control on whole period. Considering larvae biomass and feed residue on the whole period, the feed conversion of these treatments was statistically different: 3.49^{a}; 3.14^{b}; 2.74^{c}; 2.75^{c}, respectively. The crude fat content of the fresh larvae was not modified by treatments containing HMTBa, when compared to control: 27.6%; 28.9%; 30.3%; 29.0%, respectively; neither the crude protein content: 52.7%; 50.9%; 48.8%; 50.6%. Besides that, the cost of insect meal per treatment demonstrated to be lower by 20.3% when HMTBa was added in the larvae feed. Conclusion: the use of HMTBa brings high benefits in terms of performance and cost to produce BSFL insect proteins and fats. Further studies are necessary to confirm those effects and demonstrate the combined effect of different feed additives.

Preliminary data on investigation of feeding rates for black soldier fly larvae raised on waste substrate: case study with food industry waste from Czechia and Italy.

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The larvae of the black soldier fly (BSFL, *Hermetia illucens*) are powerful converters of organic waste and also valuable protein alternative as feed component for monogastric animals. The development of optimal rearing methodology is crucial to fully exploit the potential of BSFL, as it allows to optimize the use of feed and to obtain, at the end of the bioconversion process, frass that ideally only contains real feaces and not undigested food. This research aimed to verify use of the different feeding rate levels of waste-based substrates to identify the optimal one, and also determine the nutritional composition of the harvested biomass. For this purpose, two waste substrates based on local products from Czechia (cabbage leaves, apple pomace, silvery film, and brewery grains) and Italy (oversized fruits, mix fruit juice and palp, buffalo milk whey, brewery grains, rice hulls, silvery film) were fed to BSFL in 0.4, 0.6, 0.8 and 1.0 g per larva, and compared to Gainesville diet as a control. The obtained data showed that weight of harvested larvae increased with the increasing of the feeding rate, and larvae raised on both waste-based substrates reached smaller weights than those fed on control diet. Regarding chemical composition, the effect of the diet was proved to be higher than that of feeding rate. Among the diets, BSFL fed on waste products from Czechia contained the highest content of crude proteins (N × 4.67), ash, and the lowest level of lipids. The research proved the local waste from food industry may be converted to high-protein biomass. On the other hand, the further research in feeding rate design and optimization of substrate composition is highly relevant.

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The effect of temperature on the nutritional quality and growth parameters of yellow mealworm larvae (*Tenebrio molitor* L.)

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Nutritional quality of insects depends on many factors including rearing conditions. This study focusses on the effect of temperature on the basic nutritional value of Tenebrio molitor larvae. Dry matter and the ash content determined gravimetrically, lipids by the Soxhlet extraction, and total protein content by the Kjeldahl method were monitored in the yellow mealworm larvae farmed at three different temperatures (22, 25 and 28°C). Insect body length and body weight were measured as well. To evaluate deeply the protein quality amino acid profile of samples reared at all three different temperatures were determined using an amino acid analyser.

Significant differences in the body weight and length of larvae, dry matter, ash content, and lipids and the protein contents per dry matter were found depending on the temperature. There was an increasing tendency in the larvae weight (from 0.09 g to 0.15 g), dry matter content (from 30.72 g to 36.55 g) and fat per dry matter content (from 22.46 g to 36.01 g) with increasing rearing temperature. On the contrary, the clear decreasing trend (from 64.33 g to 54.41 g) was seen in case of crude protein per dry matter content. From the results of the amino acid profile, it was found that edible insects contained all essential amino acids, and the effect of temperature was not found in the case of amino acids. Limiting amino acids are sulphury amino acids, and the essential amino acid index was close to rice or rye.

The information about the effect of temperature on the larvae growth and nutritional parameters could be important for insect farmers to set the optimum rearing temperature for the purpose of the subsequent use of the insects as food or feed.

The utilization of black soldier larvae and yellow mealworms for the conversion of organic-airport-waste into fertilizer/nutrients/energy

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As the population experiences steady growth, there is a concurrent rise in the generation of waste materials. To manage the bad results of this increase, the issue of waste management should primarily emerge. Numerous laws and regulations exist pertaining to the retrieval and management of various forms of waste. Organic waste is one of them. It is known that the organic waste content possesses both nutritional and energy value. Therefore, utilizing organic wastes solely for composting purposes (conventional retrieval method) represents a substantial detriment.

The objective of this study is to offer an alternative viewpoint on the management strategy employed for organic waste, distinct from the conventional practice of compost production. A novel fertilizer strategy has been proposed, involving the segregation of nutrients and protein, with the aim of enhancing its efficacy. The presented concept involves the conversion of organic waste into various valuable outputs such as nutrients, protein, fertilizers, and energy. The selection of the airport as the sample was based on its high level of passenger activity and its significance as a populated area.

In conclusion, we suggest that it is possible to provide the energy needs of the airport in various locations by utilizing the energy obtained from the biomass produced by edible insects.

“Quality check” - Method for the conservation and storability of live larvae of the black soldier fly (BSFL)

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Live larvae offer a promising opportunity to promote animal welfare and health in pigs and poultry by, among other things, increasing animal activity and promoting natural behav-iour as an enrichment material. However, to ensure that only live and unblemished insects are fed and offered, a method of "quality check" must be established.
In the present study, the vitality, sensory and hygienic properties of the larvae were examined over a period of 9 days after harvesting to determine their shelf life.
For the experiment, the larvae were reared in 4 feeding boxes with a standard feed based on wheat bran, pig feed and water in a controlled climate chamber (28°C, 45% rH). For testing, 3 samples on each box of 14 days old, L5-BSFL were taken and immediately cooled at 8°C. To determine the larvae vitality, a score for the type and time of movement was developed, along with a sensory evaluation (appearance, odor, and consistency), which was conducted at room temperatures around 22-23°C on days 0, 3, 6, and 9 after harvest. The total bacterial count, Enterobacteriaceae, total coliforms and E.coli was also determined at the above-mentioned time points.
Immediately after harvesting, 100% of the larvae were vital. The vitality was decreased by 9% on day 3, by 17% on day 6 and by 49% on day 9 after harvest, with a general increase of tonic immobility. On day 9, 2% of the larvae were mortal. The sensory characteristics of the live larvae were very similar on day 0, 3 and 6. With longer storage, dark spots appeared more frequently on the larvae, and the odour was described as "musty, decomposed, penetrating, no longer species-specific, pungent and, slightly acidic"; the consistency of the BSFL decreased and was described as "wet, moist, damp, muddy and, flat". On day 9, the total bacterial count of BSFL was increased by 19% compared to day 0 after harvest.
The results of this study show that live black soldier fly larvae are "shelf stable" at a con-stant temperature of 8 °C for 6 days after harvesting. The storage influences the charac-teristics and shelf life of live larvae. A suitable quality check can evaluate and classify the resulting products in a grading system.

Optimal balance between protein and carbohydrate in industrial diet for black soldier fly larvae (*Hermetia illucens*)

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Black soldier fly larvae (BSFL), *Hermetia illucens*, are an efficient organism for converting organic substrates into insect biomass suitable for livestock feed. The performance of BSFLs depends on the macronutrient composition of their diet, with protein (P) and carbohydrate (C) being the two main macronutrients. This study uses a geometric approach to determine the effects of different balances between P and C concentrations in industrial diets formulated with cereals and tubers by-products from North-East of France (wheat, grain, potato, corn, beetroot). These diets were used to feed the BSFLs for seven days. The impact on the feed conversion ratio (FCR), protein and lipid content of 14-day-old larvae (14 DOL) was assessed with Agronutris strain. Seventeen oligidic diets varying in their P and C concentration were formulated, with C+P concentration ranging from 30% to 60% and C:P ratios from1:1 to 4:1.

Our results indicate that BSFL performance is more affected by C+P concentrations than C:P ratios. The best-performing diets for minimizing FCR and maximizing 14 DOL lipid content were those close to 45% C+P concentration, while 14 DOL protein content was maximized with diets containing approximately 30% C+P. These findings clarify the results already available in the literature by exploring a more restricted range of P and C. They also confirm the importance of the balanced composition of dietary protein and carbohydrate to optimize BSFL performance on an industrial scale. However, to fully understand the impact of P and C on BSFL performance, future studies should also take into account the digestibility of these macronutrients in oligidic diets.

Regional and seasonal side streams for sustainable mealworm production in the context of the Republic of Kosovo

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As the global population grows, the need for sustainable and eco-friendly protein sources rises. Insect farming emerges as a solution. This research focuses on utilizing regional and seasonal side streams from agriculture and food systems in Kosovo for mealworm production. The study aims to promote sustainable agriculture and development in this country of the West Balkans.

Dried substrates were prepared by combining agricultural by-products from Kosovo, like brewer's spent grain (BSG), yeast (BSY), apple pomace (AP), and grape pomace (GP), with wheat bran (WB). Wet side-streams of pepper, melon, and potato were added. According to a landscape analysis carried out in advance, these substrates are available in large quantities in several parts of Kosovo. Larvae of *Tenebrio molitor* L. were reared using these substrates. Growth, nutritional composition, fatty acid, amino acid, and mineral content analyses were conducted to assess substrate and larvae quality, and by-products like frass.

A substrate of WB and BSG with melon as a wet source showed the most suitable larval growth. These larvae exhibited highest growth rates, efficient feed conversion, and short development times. Analysis of feed parameters, fatty acids, amino acids, and minerals revealed variations among substrates and larvae. Mealworm frass proved valuable as organic fertilizer.

Findings highlight mealworms as an alternative protein source addressing Kosovo's environmental challenges. The optimized substrate yielded promising results for larval growth, with composition analysis showcasing nutritional characteristics of mealworms and by-products.

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