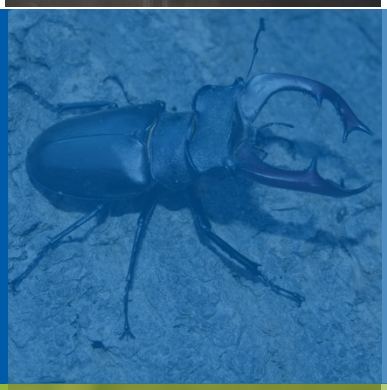

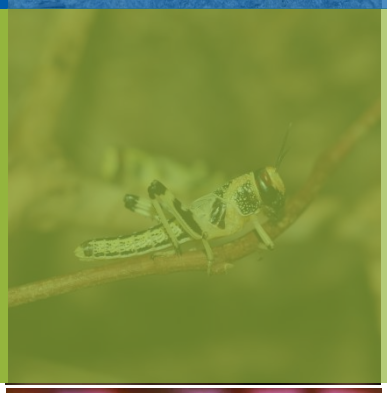

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INSECTA 2018

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INSECTA 2018
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Book of Abstracts

05th – 07th September 2018
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Prof. Dr. Andreas Vilcinskas
Prof. Dr. Marc F. Schetelig
Dr.-Ing. Sara Bußler
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Dr.-Ing. Oliver Schlüter

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Preface

Following a successful launch of the INSECTA conferences in 2015 and 2016 in Magdeburg, and the successful implementation of the INSECTA 2017 in Berlin with more than 250 participants we are delighted to have Giessen as conference venue in 2018.

Giessen is one of the main centers of insect research in Germany. Especially in view of insect biotechnology the local research group is a worldwide leader.

The INSECTA conference 2018 aims to review the state-of-the-art of insect technology and the opportunities and constraints of the use of insects as food, feed and non-food in Europe and throughout the world. This publication contains abstracts of all talks and posters presented at this conference.

Research and science-based innovation play a major role in enabling the relevant industries to address challenges faced by the sector at a global level. This conference aims to bring together companies and research innovation, helping to discover future prospects of insect technology.

Of particular interest were the developments in the European legislation, which have allowed the use of insects as feed for aquacultures since 2017, and the use of insects for food as novel foods which was uniformly regulated within the EU at the beginning of 2018.

Accordingly there exists a substantial need for discussing these developments for which the INSECTA 2018 may serve as a solid platform.

It is gratifying to see the steadily growing industry for the breeding and exploitation of insects and the equally growing research community that has been working on ideas for the future and developing new products.

Main topics of the INSECTA 2018:

Food safety for using insects
Insect production systems
Applications of insects as feed and food
Non-food applications of insects

In total, the conference has attracted 97 presentations from 19 countries. During the 2 - day conference, 7 keynote lectures and 58 oral presentations were held in 3 plenary and 12 parallel sessions. The conference featured also one additional satellite meeting and a poster session with 31 posters.

We hope that we were able to facilitate bringing together those involved in insect production, processing and consumption throughout the world, to explore the frontiers of knowledge in the art, science and engineering of all types of processing methods, monitoring technologies, and quality management systems.

A stage was provided for participants and the public to learn about the latest developments in the respective fields, to boost communication and to increase cooperation and coordination of research efforts among different research groups.

Finally, as the Chairmen of the Conference, we would like to take this opportunity to sincerely thank the contribution from the authors, the reviewers and the editorial team.

Thomas Piofczyk, Oliver Schlüter, Andreas Vilcinskas

August 2018

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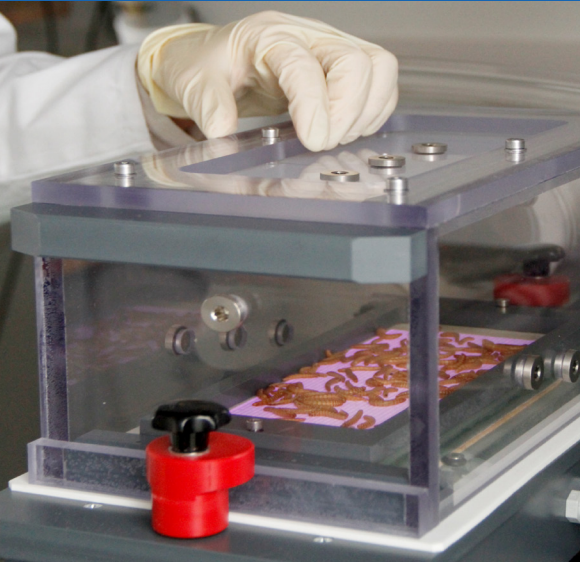
Special focus is on the systemic evaluation of the material, energy and information flows while complying a balance of quality and safety.



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+50 % in 2050 **protein gap**

2015

Crop farming

525 mio tonnes protein

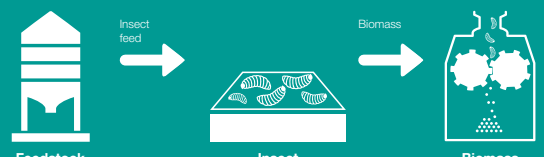
35 % food

65 % feed → food

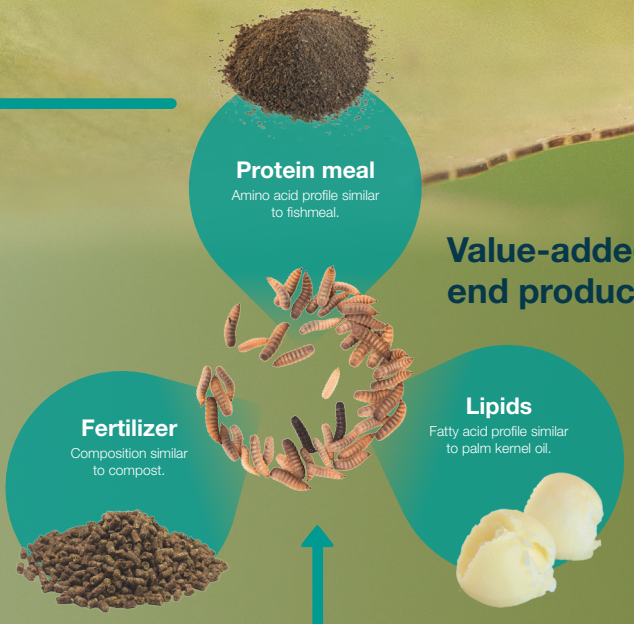
Losses & Waste

1.3 billion metric tons per year (cost-efficient raw materials)

Insect Technology



Value-added end products



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The regulatory framework for production and use of insects as feed in the EU in order to ensure the safety of the food chain: Situation and outlook

Wolfgang Trunk

DG SANTE / Animal Nutrition, Veterinary Medicine, Brüssel, Belgium
Corresponding author: Wolfgang.TRUNK@ec.europa.eu

Insects as feed and food are a quite young areas the EU. The European Commission started in 2014 with a strategy paper on the "Use of insects as food and feed in the EU". Subsequently, several milestones have been reached, starting with the EFSA`s risk profiling in 2015 and several adaptiations of the EU feed and food safety legislation. The presentation will build up on the principles of European Food safety legislation to be applied to insect production and use, and expand on these new Regulations. Thirdly, it would give an outlook about what might be next regulatory steps to accompany a solid development of the sector with appropriate safety rules.

Adaptive strategies of insects to challenging environments

Heiko Vogel[1], Shantanu Shukla[1], Martin Kaltenpoth[2], David G. Heckel[1], Andreas Vilcinskas[3,4]

[1] Group Leader, Department of Entomology
Max Planck Institute for Chemical Ecology, Jena, Germany
[2] Institute of Organismic and Molecular Evolution,
Johannes Gutenberg University of Mainz, Mainz Germany
[3] Institute of Insect Biotechnology, Justus-Liebig-University of Giessen,
Giessen Germany
[4] Department Bioresources, Fraunhofer Institute for Molecular Biology and Applied Ecology,
Giessen, Germany

Corresponding author: hvogel@ice.mpg.de

The tremendous evolutionary success of insects is at least in part owed to microbial symbionts which help them to explore novel ecological niches such as the ability to utilize unusual diets. Selective pressures from xenobiotics, nutrient limitations and natural pathogens may contribute to driving insects to adapt towards and enable exploitation of these diets. A highly efficient digestive mechanism is required by such insects to support rapid nutrient assimilation and biomass conversion during development. Furthermore, an effective immune system is required to cope with elevated levels of pathogenic microorganisms.

An intriguing example of food preservation and controlling food-associated microbiomes are the burying beetles (*Nicrophorus* spp) that have evolved to occupy a unique ecological niche by reproducing on small vertebrate cadavers buried in the soil. Carrion-feeding insects that breed on decaying carcasses must overcome the challenges arising from competing microbes, which decrease its nutritional value and render it unpalatable or even toxic to animals. We show that the burying beetle modifies the microbial communities of carrion, since beetle-tended carcasses showed no signs of degradation and supported the growth of the beetles' gut microbiota, including the yeast *Yarrowia,* in a biofilm-like matrix. In contrast, untended carcasses showed visual and olfactory signs of putrefaction and supported the growth of endogenous and soil-originating microbial decomposers. The beetles and their microbiome thus coordinate a specialized adaptive strategy of carrion management, enabling them to preserve carrion quality and support larval growth on a challenging resource such as carrion.

Insect meal as a source of protein in animal nutrition

Klaus Eder, Robert Ringseis

Institute of Animal and Nutritional Physiology,
Justus-Liebig-University of Giessen, Giessen, Germany

Corresponding author: Klaus.Eder@ernaehrung.uni-giessen.de

According to predictions of international organizations, the global population will rise to around 10 milliards of people in 2050. It is estimated that the global requirement of foods will increase by 70% and the extent of animal production will increase by 50% until that time. These massive increases in food requirement and animal production will lead to a strong increase in the global demand of protein. Therefore, the development of strategies for a sustainable production of alternative sources of protein has great priority. Protein rich meals from insects are promising candidates for the supply of protein for humans and farm animals in future. However, for the use of insect meals as a source of protein in farm animals, one prerequisite is that animal performance achieved by feeding insect meal is comparable with that reached by feeding conventional sources of proteins such as soybean meal. Moreover, potential safety risks for consumers resulting from feeding insect meals of meat must be excluded. Several studies in broilers have already shown that insect meals are valuable sources of food protein, with a biological value similar to that of soybean meal. Few studies indicate that insect meals might be also suitable sources of proteins in pig nutrition. However, the effects of insect meals on metabolism of farm animals and potential risks for consumers deriving from potential adverse compounds occurring in insect meals penetrating into meat of animals have not yet been investigated. Therefore, investigations on safety aspects, regarding both animal and human health are required.

Monitoring the microbiota during industrial insect rearing to unravel feed and food safety

Leen Van Campenhout

KU Leuven, Department of Microbial and Molecular Systems, Lab4Food, Technology Campus Geel, Geel, Belgium

Corresponding author: leen.vancampenhout@kuleuven.be

A prerequisite for insects as a sustainable protein source for humans and animals is their food safety, which implies the absence of physical, chemical and microbial risks upon consumption. Risk factors can either be introduced via the substrate or via the rearing environment (cages, crates, personnel, air, surfaces, equipment). In order to perform a microbial risk assessment, insight is needed in the microbial load (contamination levels as well as types of bacteria, yeasts, moulds and viruses present) of insects during rearing in an industrial environment. In the last few years, several industrial insect producers allowed sampling in their facility to monitor the microbial community of the insects and their substrate throughout the life cycle. The insect species investigated in this way include the lesser mealworm (*Alphitobius diaperinus*), the cricket (*Gryllodes sigillatus*) and the black soldier fly (*Hermetia illucens*). Data collection could reveal whether food pathogens were present or not, where micro-organisms can potentially be introduced or eliminated during rearing and processing, and how the substrate can affect the microbial load of the insects and vice versa. Even though no food pathogens were found in the cycles studied, the question remains whether transmission of a pathogen from substrate to insect would occur in the event that the pathogen would be present in the substrate. For black soldier fly larvae, several pilot scale and industrial locations were included in the study and could be compared with respect to the bacterial community. The possibility to control the larval gut microbiota may also allow to improve yield and growth rate and may entail new opportunities for valorisation. The commitment and contribution of insect producers in this type of research, not only from the microbial but also from the chemical point of view, is indispensable, as it can stimulate progress in the whole insect sector, e.g. by supporting the establishment of an appropriate legislative framework.

The greater wax moth *Galleria mellonella* in the study of microbial pathogenesis and antimicrobial drug discovery

Eleftherios Mylonakis

Alpert Medical School of Brown University Rhode Island Hospital, USA

Corresponding author: emylonakis@Lifespan.org

Invertebrate model hosts provide ethical, logistical and budgetary advantages. The facile inoculum delivery and handling of the insect *Galleria mellonella* make it a desirable model for the study of bacterial and fungal pathogens. Using this model, various pathogenic microbes, such as Staphylococcus aureus, Pseudomonas aeruginosa, Listeria monocytogenes, Helicobacter pylori, have been studied and many novel virulence genes have been identified. Notably, this insect pathosystem also offers the opportunity to examine the physical barriers, cellular mechanisms and molecular elements of the host response to human pathogens and offers a simple strategy to evaluate the efficacy and toxicity of antimicrobial agents, even in sub-MIC concentrations. Importantly, this host allows for complex studies such as the modeling of biofilms and the relationship between antibiotic drug resistance and pathogenesis, the study of antimicrobial compounds, peptides and probiotics, and the evaluation of complex treatments including photodynamic therapy. Overall, the use of *G. mellonella* as a model host to elucidate microbial pathogenesis and identify novel drugs and therapies has been well appreciated over the past years. This model host allows for the rapid, inexpensive, and reliable study of pathogenesis, the identification of virulence factors and the testing of potential antimicrobial compounds. Such studies and large pathogenesis screens can utilize *G. mellonella* as the only host, or implement a multi-host approach that includes diverse model hosts, such as nematodes, *G. mellonella*, and mice.

Oral Presentations

Session 1 – 4

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Food safety for using insects

Topic 2:
Insect production systems

Topic 3:
Applications of insects as feed and food

Topic 4:
Non-food applications of insects

Legal challenges and opportunities for insects as novel food

Nicolas Carbonnelle

Bird & Bird LLP, Brussels, Belgium

Corresponding author: nicolas.carbonnelle@twobirds.com

Since 1 January 2018, a new legal framework applies to novel foods, namely Regulation (EU) 2015/2283. This legislative text is of utmost importance for companies placing insects as food on the EU market.

Before the adoption of the new novel food regulation, there was legal uncertainty on the regulatory classification of edible insects. Except for Regulation 834/2007/EC on organic production, no mention was expressly made to whole insects as food in the European legislation. Considering this legal gap, insects and insect-based food were considered by most to fall within the scope of Regulation (EC) No 258/97 on novel foods, although this qualification was far from being undisputed and not consistently implemented in EU Member States. The classification of insects as novel food has been clarified through the adoption of Regulation (EU) 2015/2283, which repeals and replaces Regulation (EC) No 258/97. Recital 8 of the new regulation expressly states that “it is appropriate to review, clarify and update the categories of food which constitute novel foods. Those categories should cover whole insects and their parts”. Following this recast of the applicable legislation, all edible insects can be deemed to fall within the novel food category under article 3, 2 (a), (v) that encompasses “food consisting of, isolated from or produced from animals or their parts –unless an applicant can demonstrate that an insect he seeks authorisation for would have been consumed to a significant extent on the EU territory before 15 May 1997. The new legal framework on novel food thus clarifies the legal status of whole insects and paves the way of insects to the EU consumers' diet.

The novel food status of edible insects implies that they are subject to safety assessment and pre-market approval before being placed on the market. Regulation (EU) 2015/2283 provides two different procedures for the placing of edible insects in the European market, one of which is specifically designed for products with a history of safe consumption as food in non-EU countries.

In both procedures, i.e. the ordinary procedure and the notification procedure, the applicant is required to submit a set of information to the Commission, that may involve the European Food Safety Authority (EFSA) in the food safety assessment. The procedures organized under Regulation (EU) 2015/2283 present specific features that introduce important novelties into the regulatory landscape of novel foods, including the generic character of the authorizations granted in accordance with the regulation and –as an exception thereto- a regime of data protection that allows applicants to benefit from a certain degree of market exclusivity under certain conditions.

The so-called "notification procedure" for traditional foods from non-EU countries is intended to be faster than the ordinary authorisation procedure, however some limits are attached to this procedure. In particular, applicants cannot obtain data protection of the information submitted in the notification dossier, meaning that – the authorisation being generic – the first applicant cannot secure a competitive advantage towards the other insects producers.

This oral presentation will analyze the procedures provided for under the new novel food regulation, and will comment on the first months of implementation of this recast legal framework.

Microbiological parameters in national European guidelines for edible insects – challenges for the future

Nils Th. Grabowski, Birte Ahlfeld, Corinna Kehrenberg

Hannover University of Veterinary Medicine, Foundation, Institute for Food Quality and Food Safety, Hannover, Germany

Corresponding author: Nils.Grabowski@tiho-hannover.de

An important aspect of public health is monitoring the microbiological quality and safety of the foodstuffs. Regulation (EU) 2073/2005 established microbiological criteria for many foodstuffs. They apply at two stages, i.e. process hygiene criteria" and "food safety criteria". For edible insects no such official EU criteria exist, a gap filled by many national European guidelines dealing with edible insects. They sometimes differ among countries in number parameters and evaluation schemes. Food hygiene criteria (typically salmonellae, Listeria monocytogenes, Escherichia coli etc.) are generally met, as these pathogens are usually inactivated even with relative mild heat treatments, e.g. pasteurization. Regarding process hygiene criteria, more differences occur, and some thresholds are typically not met. The main reasons are:

a) these parameters/evaluation schemes were not genuinely developed from insects, but from minced meat and/or seafood, and a direct comparison is questionable;

b) Each insect species hosts an individual microbiome, and each species' microbiome reacts differently towards heating;

c) Processing also affects the microbial significantly (e.g. deep-fried vs. dried).

The present sets of criteria are based on ordinary (vertebrate) foodstuffs. The insect microbiome is different, and some of their bacteria and fungi may cause disease in humans, typically YOPIs. However, there is no report on a human patient having acquired a zoonosis after consuming heated insects.

As the EU started accepting applications for insects as novel food, the next step will be to develop corresponding quality and safety parameters. These should be developed considering the species and the processing/product type rather than presenting "one-size-fits-all" criteria.

Microbial characterization of the grasshopper *Ruspolia differens* Serville, a wild harvested edible insect in Uganda

Geoffrey Ssepuuya[1], Enya Wynants[1], Christel Verreth[2], Sam Crauwels[2], Bart Lievens[2], Johan Claes[1], Dorothy Nakimbugwe[3], Leen Van Campenhout[1]

[1] KU Leuven, Department of Microbial and Molecular Systems (M2S), Lab4Food, Technology Campus Geel, Geel, Belgium
[2] KU Leuven, Department of Microbial and Molecular Systems (M2S), Laboratory for Process Microbial Ecology and Bioinspirational Management (PME & BIM), Campus De Nayer, Sint-Katelijne-Waver, Belgium
[3] Makerere University, Department of Food Technology and Nutrition, CAES, Makerere Hill Road, Kampala, Uganda

Corresponding author: geoffrey.ssepuuya@kuleuven.be

The grasshopper *Ruspolia differens*, 'nseenene' in Luganda, is an edible insect and a commercial delicacy in Uganda. It swarms bi-annually (March - May and November - December), majorly in the geographical areas of Masaka, Kampala and Fort Portal. Raw grasshoppers are sold on local markets, either as plucked (i.e. wings, legs and antennae removed) or whole insects. The microbial quality and safety of *R. differens* have not been characterized so far. This study aimed at establishing its intrinsic properties and bacterial community composition, and how these parameters are influenced by its sourcing geographical area, swarming season and plucking treatment. The intrinsic properties observed i.e., water activity (0.975 - 0.987), moisture (43.96 - 61.32%) and pH (5.75 - 6.60), suggest that the insects can support the growth of a wide diversity of microorganisms. The high counts (log cfu/g) of total aerobic microbes (8.38 - 9.41), Enterobacteriaceae (6.89 - 7.83), lactic acid bacteria (7.99 - 9.11), total aerobic spores (3.75 - 4.87) and yeast and moulds (5.77 - 7.12) can cause microbial spoilage. Metagenetic analyses targeting the prokaryotic 16S ribosomal RNA gene yielded 1793 species-level Operational Taxonomic Units (OTUs) belonging to 24 phyla. In particular, members of Acinetobacter, Buttiauxella, Lactococcus, Staphylococcus and Undibacterium represented the most abundant OTUs. Further, the identified eight genera (Acinetobacter, Bacillus, Buttiauxella, Campylobacter, Clostridium, Neisseria, Pseudomonas, and Staphylococcus) harbouring potential pathogens can expose consumers to food borne illnesses. Sourcing geographical area, swarming season and plucking treatment significantly influenced microbial counts and bacterial diversity.

A new mathematical approach to predict optimal environmental conditions in the larvae growth process

Adi Jonas-Levi, Tamar Zemach

Tel-Hai College, Upper Galilee, Kiryat Shmona, Israel

Corresponding author: adijon@gmail.com

Growing insects for food, feed or waste decomposition has become a popular subject in recent years. The physiological condition and growth rate of insects are influenced by many environmental factors. Usually, experiments are used to determine the duration of the growth process and the total product mass, under specific constant growth conditions. However, under varying environmental conditions and non-permanent substrate composition the ability to anticipate these parameters experimentally in advance is challenging. In addition, to optimize the processes, a-priori evaluation of the growth and harvest parameters is requested. Performing such nutrition experiments for a variety of possible conditions is expensive and it requires time, space, suitable infrastructure and workforce. However, mathematical analysis of the problem parameters and environmental conditions might reduce dramatically the number of the requested biological experiments and predict the behavior of the process. In this study, we formulate a mathematical model for various sets of independent variables (the composition of proteins, carbohydrates, fats, minerals, fiber, ammonium, temperature and humidity of the growth medium) and show how this model can predict a-priori the larvae mass, the time needed to reach the maximal mass, and the survival rate for various problem configurations. We also perform comparisons with previously reported experimental data which was obtained from experiments done under controlled conditions using ad libitum iso-caloric diets. And show that the laboratory data are fairly well reproduced by the theoretical results.

The effects of temperature on growth rate, feed conversion efficiency and protein content of Yellow mealworm (*Tenebrio molitor*)

Jonas Lembcke Andersen, Dahl Bjørge Julie, Johannes Overgaard, Hans Malte, Ida Elisabeth Berggreen, Natasja Gianotten, Lars-Henrik Heckmann

Danish Technological Institute, Bioengineering and Environmental Technology, Aarhus C, Denmark

Corresponding author: jlan@teknologisk.dk

Optimization of growth rates and protein content is of great importance for the future of the insect industry. Temperature is one of the most important abiotic factors for insect growth rates and is often tuned to maximize the growth. The optimal growth rate and high protein (or fat) content are both very important parameters to ensure a viable business model for insects as feed and food. In the presented data *Tenebrio molitor* larvae were tested at range of temperatures from 15.2 to 39.0°C and growth rate and macro nutrient content were collected (as well as oxygen consumption, CO^2 production.) *Tenebrio molitor* has an optimal growth rate around 31°C, however, both the final protein content and the feed conversion efficiency were higher at temperatures below 31°C. This data suggests that optimal feed conversion and optimal protein content are not found at the temperature with the highest growth rate. Temperature has large and independent effects on growth rate, energy assimilation efficiency and protein/lipid content. Thus, depending on the business model in question it is important to consider, the most optimal temperature for protein (or fat) output. In summary, temperature is one of the most critical parameters to control in commercial insect rearing regardless of the producer's desire to optimise either production speed, production efficiency or product quality.

Safety and productivity assessment of *Hermetia illucens* larvae reared on solid aquaculture waste

Eric Schmitt[1], Ram K. R. Shanmugam[2], Johan Johansen[3], Raymond Leushuis[1], Erik-Jan Lock[4], Aman Paul[1], Joop Van Loon[2]

[1] *Protix B.V., R&D, Dongen, Netherlands*
[2] *Wageningen University, Laboratory of Entomology, Wageningen, Netherlands*
[3] *Salten Havbrukspark AS, Nygårdsjøen, Norway*
[4] *Institute of Marine Research, Bergen, Norway*

Corresponding author: aman.paul@protix.eu

The use of insects as feed for animals is particularly attractive because of their capability to consume a wide variety of organic streams. However, careful attention must be paid to safety aspects while exploring novel feeds for insects. This presentation provides a case study for this process. Aquaculture is a rapidly growing source of animal protein that leaves a substantial environmental footprint through the production of feed inputs and through waste streams produced in fish farming. A circular system that can upscale aquaculture wastes to fish feed could reduce the environmental impact of the production system. Research on *Hermetia illucens*, the black soldier fly, has shown that larvae from this species is both suitable as feed for aquaculture species and is capable of growing on a wide variety of feeds including feces. Therefore, it would appear that *Hermetia illucens* is an attractive solution for closing the loop between aquaculture waste and feed. However, aquaculture waste streams are known to contain high levels of heavy metals, which have been shown to accumulate in the larvae of *Hermetia illucens*. Results from tests performed with the solid fraction of a high volume aquaculture waste stream will be presented from a safety standpoint with a focus on demonstrating how feed experiments can be linked to animal feed regulations.

A peptidase from the bug *Spilostethus pandurus* for food processing

Wendell Albuquerque, Martin Rühl, Martin Gand, Holger Zorn

Justus Liebig University Giessen,
Food Chemistry and Food Biotechnology, Giessen, Germany

Corresponding author: holger.zorn@uni-giessen.de

Peptidases play an important role in e.g. cheese production, tenderizing of meat, and clarification of beverages, where turbidity caused by protein aggregation is a common cause of instability and impairment of product quality. Currently, there is a lack of suitable peptidases especially for applications in wine production. At the same time, food protein hydrolysates have a wide range of applications, as providing fortification agents in beverages and also as predigested ingredients for enteral/parenteral nutrition. In this regard, the need to find viable sources of peptidases with high catalytic activity and specificity to particular substrates has become a challenge and scientific efforts have been done to provide such alternatives.

Insects are organisms well-adapted to diverse harsh environments, a fact which directly induces their gene-expression system to produce a broad set of enzymes. Therefore, various species are considered as interesting sources of biological active compounds. For their ability to feed on the most diverse nutrient sources, insects are reported as a great source of peptidases, which mainly originate from their salivary glands and gut.

In the present study, crude extracts from the bug *Spilostethus pandurus* have been obtained through the subsequent steps of maceration, buffer extraction and centrifugation. Zymograms for preliminary screening were performed by using the substrates casein, gelatin, albumin and grape proteins (wine haze), confirming the hydrolysis capability of the extracts against the proteins assayed. Purification steps have been performed through a Fast Protein Liquid Chromatography (FPLC) system using ion-exchange (DEAE-FF column) and size-exclusion (Superdex 200 column) chromatography. The degree of purification was confirmed by SDS-PAGE. The enzyme inhibition by PMSF identified the target enzyme as a serine-peptidase. The purified peptidase from *Spilostethus pandurus* showed high activities and the capability to degrade various substrates. Further applications such as clarification of wine (as an alternative to the use of bentonite), meat tenderizing and debittering processes will be considered.

Sustainable production of high quality feed from animal (*Hermetia*) and plant (*Lemna*) sources

Herwig O. Gutzeit, Ariane Müller
TU Dresden, Faculty of Biology, Dresden, Germany
Corresponding author: herwig.gutzeit@gmail.com

The increasing worldwide demand for protein has led to the expansion of soy and other monocultures at the expense of rain forests and other valuable ecosystems. The growing human population and the increasing consumption of beef and pork results in a “protein gap” that needs to be closed using alternative sources of protein with little use of arable land.

Insect mass cultures offer an alternative. The production of larvae of the soldier fly *Hermetia illucens* fed with organic waste can be upscaled so that a significant contribution to animal feed is, in principle, possible. The physiological requirements of different livestock species may necessitate the separation and processing of the larval protein and lipid constituents. For example, the *Hermetia* larvae are too rich in lipids to be suitable fish feed so that the lipids need to be (partially) removed. We intend to circumvent this problem by the preparation of feed pellets consisting of *Hermetia* larvae and homogenates of duckweed. This plant is characterized by rapid cell divisions and the culture is simple. Duckweed (e.g. *Lemna* or other suitable species) is rich in protein (in the order of 35% dry weight) and sugars. The lipid content is low but valuable omega-3 fatty acids are present. By varying the ratios of *Hermetia* to *Lemna* homogenates feed pellets can be prepared that meet the specific requirements of different species. A container will be designed that allows the culture of duckweed in an efficient way using a minimum of space. The liquid manure that is produced by the *Hermetia* culture is used to stimulate growth of the *Lemna* cultures. In this closed-circle production no waste products will be generated. The various parameters that are relevant for maximal plant growth are being studied in a current research project.

Our approach combines the advantage of animal- and plant-derived feed and offers maximal flexibility concerning the desired molecular constituents for species-specific animal feed. The products that we intend to develop should have a health benefit for the animals (e.g. poultry or fish) compared to conventional feed containing soy or fishmeal. To this end we analyse natural constituents in *Hermetia* and *Lemna* homogenates and pay particular attention to antimicrobial peptides (AMPs) and small organic bioactive constituents with suspected health-promoting properties.

The aim of the project is to produce feed that helps to maintain the health of animals in factory farming and, in addition, contributes to the conservation of valuable ecosystems worldwide.

Acknowledgements: This work is carried out in close collaboration with Jens Liebscher, Kai Kreller and Dennis Hluschi of the Bio.S Biogas GmbH. The optimization of duckweed cultures is carried out in collaboration with the TERRA URBANA GmbH.

A house for the critters: Bringing insects into people's kitchens

Andreas Walter[1], Thomas Klammsteiner[2], Carina Desirée Heussler[2], Heinrich Pan[3], Markus Schermer[4], Heribert Insam[2]

[1] *MCI-Management Center Innsbruck, Biotechnology & Food Engineering, Innsbruck, Austria*
[2] *University of Innsbruck, Department of Microbiology, Innsbruck, Austria*
[3] *Fablab, Spielraum, Innsbruck, Austria*
[4] *University of Innsbruck, Department of Sociology, Innsbruck, Austria*

Corresponding author: csaf2555@uibk.ac.at

Insects have played a vital role in the history of mankind, especially in Asia, Africa and South America. In some cultures, they are still used in traditional medicine. Nowadays, even in regions with no history of insect-use, their (re) appearance is on the rise. This modern trend ranges from combining entomophagy with western cuisine to large-scale industrial applications. In our citizen science project entitled "Six-legged Livestock: Rearing Black Soldier Fly on biowaste", we want to contribute to free the minds of prejudice and aversion against insect life forms in a society where bugs usually have a bad reputation as pests or vectors for disease. The increasing alienation from nature slowly results in overseeing the key role that insects play in our lives. In cooperation with a local citizen-oriented fabrication laboratory (FabLab) we organize a series of workshops for various target audiences, during which we will build small house-like rearing boxes using laser cutter and numerically controlled (CNC) mill technology. The hardboard houses will enclose a leak-tight container in which black soldier fly larvae can be raised on organic kitchen waste. Leachates are collected in a detachable glass jar for further use as e.g. fertilizer, and larvae, once on the lookout for a dry pupation niche, can escape the container via ramps. The citizen scientists will contribute to our research by providing data on the type and amounts of waste added to the population as well as the produced larval biomass.

Species differentiation of insect protein in compound feed using proteomics approach

Ikram Belghit[1], Josef D Rasinger[1], Erik-Jan Lock[1], Marc Berntssen[1], Olivier Fumière[2], Marie-Caroline Lecrenier[1], Marc Dieu[1], Magnus Palmblad[1]

[1] *Institute of Marine Research, Bergen, Norway*
[2] *Agricultural Product Valorisation Department, Gembloux, Belgium*

Corresponding author: ikram.belghit@hi.no

Aquaculture is amongst the most efficient ways to produce animal protein for human consumption, and this sector is expected to continue to grow worldwide. Inclusion of novel protein sources, like insect meal, may help to mitigate the expected scarcities of feed resources. Indeed, insect ingredients hold a great potential as a source of nutrients for different fish species. In addition, insect production on organic side-streams can valorize many types of organic materials by producing protein for aquafeed. However, considering as animal protein, insect should comply with legal constraints and guarantee the safe use in fish feed ingredients. Furthermore, there is a need for detection of the insect ingredients identity used in aquafeed. In the current study, we used proteomics tools, for the detection and differentiation between eighteen different insect meal samples, from the species *Hermetia illucens*, *Tenebrio molitor*, *Alphitobius diaperinus* and *Acheta domesticus*, belonging to the *Arthropoda*, *Coleoptera* and *Orthoptera* phyla. The gel-free shotgun proteomics approaches in combination with direct spectral comparison were able to differentiate specifically the insect meal samples, according to the taxonomic classification of the insect species. Thus, this biological fingerprinting methodology is a useful tool for species specific discrimination of insect's protein.

Mycotoxin detection in food- and feed-stuffs by living insects as rapid test

Marlen Albrecht

Pilot Pflanzenoeltechnologie Magdeburg e.V., Magdeburg, Germany

Corresponding author: albrecht.marlen.sr@gmail.com

Mycotoxins are secondary metabolic products of mold fungus. They can be carcinogenic, mutagenic, teratogenic and immunosuppressive. In addition to foodstuffs, feedstuffs can also be affected by mold fungus and thus have an impact on meat and milk production.

The infestation by mycotoxins is an unavoidable risk because their formation is weather-dependent and effective prevention is thus impossible. Therefore, most countries have introduced guidelines or limits for mycotoxins.

The routine detection of mycotoxins is currently being carried out using residue analytical methods (HPLC) or rapid tests (ELISA, bio-assay). Both variants require an external laboratory. The targeted procedure will save time by its on-site character, but cannot replace the quantitatively secured HPLC method for positive-tested samples.

The actors in the animal feed industry are thereby able to better meet their own control requirements with this faster novel device.

The biggest advantage of the new procedure are the low costs. Because of that the operating risk reduces to a considerable degree, the sample density can be increase and makes an important contribution to the improvement of feed safety.

As a sensor, living insects will be used, since these can detect much more reliably volatile mycotoxins with their sensitive olfactory sensor system, compared to e.g. synthetically-produced polyclonal antibodies of conventional ELISA tests. These tests tend to lead to nonspecific cross-reactions, which lead to false results. All device components as well as attractant and scent extracts necessary for a safe measurement have to be developed within the project and tested under practical conditions.

Transcriptomic and proteomic analysis of the bombardier beetle defensive gland to identify proteins mediating the explosive defense reaction

Nicolai Rügen[1], Heiko Vogel[2], Natalie Wielsch[2], Miray Tonk[3], Andreas Vilcinskas[3]

[1] *Fraunhofer Institute for Molecular Biology and Applied Ecology, Bioresources, Giessen, Germany*
[2] *Max Planck Institute for Chemical Ecology, Entomology, Jena, Germany*
[3] *Justus Liebig University, Institute for Insect Biotechnology Giessen, Germany*

Corresponding author: nicolai.ruegen@ime.fraunhofer.de

Insects display a variety of strategies to protect themselves from predators and food competitors. The defense mechanisms involving repellent chemicals are considered to be among the most efficient. Bombardier beetles (Carabidae), when stimulated or threatened by predators, display a unique chemical defense reactions. The beetles respond to a threat with paired pygidial defensive glands and instantly release, within 50 to 200 ms, as a spray, of a variety of defense chemicals. The latter are produced in two separate explosion chambers and released through a spray nozzle, located at the end of the abdomen, which precisely targets the threatening predator. The effectiveness and complexity of this defense system has therefore drawn attention for research and application studies. However, the genes involved in bombardier beetles' chemical defense are unknown, yet. We performed a combined transcriptomic and proteomic analysis of the defense gland to identify the enzymes mediation the explosive reaction. Ultimately, such enzymes could have potential for applications in industrial biotechnology.

The gelling potential of larvae and pupae of honey bee (*Apis mellifera*)

Maryia Yu Mishyna[1,2], Jianshe Chen[1], Jean-Jacques Itzhak Martinez[3,4], Ofir Benjamin[2]

[1] *Zhejiang Gongshang University, School of Food Science and Bioengineering, Hangzhou, China*
[2] *Tel Hai College, Food Science Department, Upper Galilee, Israel*
[3] *Tel Hai College, Animal Science Department, Upper Galilee, Israel*
[4] *MIGAL, Animal Ecology and Biodiversity Lab, Kiryat Shmona, Israel*

Corresponding author: maryia.mishyna@gmail.com

Honey bee (*Apis mellifera*) is a beneficial insect mainly associated with honey production and pollination, but honey bee brood can also be consumed raw, boiled, or roasted in several Asian and central America countries (RAMOS-ELORDUY *et al.* 1997, YHOUNG-AREE *et al.* 1997, ZHI-YI 1997). Recent studies have demonstrated that larvae and pupae of honey bee have a high protein content, a well-balanced composition of fatty acids and a significant amount of iron and zinc (GHOSH *et al.* 2016). However, the functional properties of honey bee brood are still poorly studied; so the aim of this research was to evaluate the emulsifying, foaming and gelling properties of honey bee larvae and pupae and to elucidate the nature of protein interactions occurring during heat gelation. Honey bees were reared naturally on pollen and on nectar with an addition of sucrose; after harvesting they were separated into larvae and pupae, mixed at the ratio 1:1, and freeze-dried. The content of protein (22%), fat (20%), carbohydrates and the composition of amino acids were determined for raw powder of honey bee brood. Foaming capacity (5.8%) was found significantly lower than that of whey proteins (98.3%), and 33.3% of foam remained after 120 min of incubation. Emulsifying capacity and stability of honey bee brood were determined at 20.8 and 34.3% respectively. The highest coagulation of soluble proteins was observed at pH 5 and 7 after heating at 85 C, while at the lower temperature (55 C) the coagulation at the isoelectric point (pH 5) was significantly higher, than in pH 3, 7, and 9. The minimum range for protein concentration to form a gel from raw powder varied from 1.1 to 2.4% at pH 7 and 3, respectively. The significant increase in a surface-weighted mean of protein aggregates after heating was observed at pH 5, 7, and 9. Changes in protein hydrophobicity, charge, amount of exposed and buried -SH groups demonstrated the dominance of either covalent or non-covalent intermolecular interactions led to aggregation in different pH. The gel hardness increased at more alkaline pH, while springiness showed no difference. Finally, gel microstructure at different pH have been visualized by SEM microscope and the size of pores was calculated. Our results demonstrated the high potential in using honey bee brood to form a gel under different pH and temperature conditions. This can lead to more future formulation of an edible insect based gel products as in the case of honey bee.

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Recombinant production of insect derived antimicrobial peptides in stably transformed *Drosophila melanogaster* S2 cells

Jan Zitzmann, Tobias Weidner, Peter Czermak

Technische Hochschule Mittelhessen - University of Applied Sciences, Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany

Corresponding author: jan.zitzmann@lse.thm.de

Antimicrobial peptides (AMPs) from insects are valuable resources for pharmaceutical industry and can serve as leads for the development of novel antibiotics. To access this potential an effective recombinant expression process is mandatory, which includes the selection of a suitable expression host as well as process optimization during scale up. Here we describe the recombinant production of AMPs derived from the greater wax moth *Galleria mellonella* or the ladybug *Harmonia axyridis* using stably transformed *Drosophila melanogaster* S2 cells. Based on the polyclonal population that was obtained after transfection, we isolated highly productive single cell clones by limiting dilution and achieved a two to sixfold increase in productivity. Further optimization on the cellular level included a statistical planned screening to determine optimal conditions for induction of the employed Metallothionein promoter. The online measurement of the cell suspensions dielectric properties and turbidity enabled an efficient process control and monitoring of the cells physiological status. Based on this information, 25 mg of the AMP was expressed at the 1^{-L} bioreactor scale in batch mode. Current focus of this work is the employment of tangential flow filtration to enable efficient perfusion processes. Using a short perfusion run at the bench scale already increased the final protein yield to 130 mg using essentially the same equipment as for batch culture. Finally the functional AMPs were recovered by affinity chromatography and the antimicrobial properties against model strains of Escherichia coli were tested, indicating the successful isolation of active peptides.

Satellite meeting at the Insecta 2018

6. September 2018; New Chemistry / Aula, Gießen

2nd Gießen Symposium for Insect Biotechnology & Bioresources

Resistance and sequestration: drivers of insect-plant coevolution

Georg Petschenka

Justus-Liebig-Universität Giessen, Institut für Insektenbiotechnologie Giessen, Germany

Corresponding author: Georg.P.Petschenka@agrar.uni-giessen.de

Natural history collections form the world's basis for taxonomic, systematic, and faunistic research. While it has long been realized that genetic information (DNA) for phylogenetic reconstruction can be frequently retrieved from museum specimens, the potential for analysis of contained natural products has been largely overlooked. Here we show that especially dried insect specimens provide a rich resource of chemical information, which can be used to reconstruct the ecology of species and may furthermore serve as a resource for the discovery of novel natural products. The seed bug *Spilostethus saxatilis* absorbs high concentrations of colchicine and related alkaloids from meadow saffron (*Colchicum autumnale*) to defend against predators such as birds. While *S. saxatilis* appears to feed broadly on seeds of various plant species it was not known if it is associated with *Colchicum* obligatorily. To test this hypothesis we developed a non-destructive approach to extract natural products from dry museum specimens and analyzed >25 *S. saxatilis* museum specimens from over 10 countries (Europe and North Africa) using HPLC. All specimens contained *Colchicum*-alkaloids, sometimes in very high amounts, proving that each individual insect accessed *C. autumnale* during its lifetime. Remarkably, we obtained alkaloids from specimens, which were more than 100 years old. Similarly, in an additional approach involving museum specimens, we found two further seed bug species to sequester pyrrolizidine alkaloids. Here, specific structural features of the alkaloid molecules revealed information about the host plant families used by the insects. In a third study based on the extraction of museum specimens, we focused on defensive toxins (bufadienolides) from European and African firefly species (Lampyridae). Here, our approach even revealed compounds, which were previously unknown. Our findings highlight the tremendous value of natural history collections as a treasure of chemical information to analyze natural history traits and as a valuable resource for natural product research.

Complex relationship between the pea aphid *Acyrthosiphon pisum* and its bacterial symbiont *Serratia symbiotica*

Marisa Skaljac[1], Phillipp Kirfel[1], Jens Grotmann[1], Heiko Vogel[2], Natalie Wielsch[2], Sanja Mihajlovic[1], Andreas Vilcinskas[1,3]

[1] *Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Bioresources Project Group, Giessen, Germany*
[2] *Max-Planck Institute for Chemical Ecology, Jena, Germany*
[3] *Institute for Insect Biotechnology, Justus Liebig University of Giessen, Giessen, Germany*

Corresponding author: marisa.skaljac@ime.fraunhofer.de

Aphids are economically important pest insects that damage plants by phloem feeding and the transmission of plant viruses. Their ability to feed exclusively on nutritionally-poor phloem sap is dependent on the obligatory symbiotic bacterium *Buchnera aphidicola*, but additional facultative symbionts may also be present, the most common of which is *Serratia symbiotica*. The genus *Serratia* releases an arsenal of virulence-associated enzymes, so we hypothesized that *S. symbiotica* may produce proteases that act alongside aphid enzymes to digest phloem sap proteins. Using molecular tools, including fluorescence *in situ* hybridization, we found that *S. symbiotica* colonizes the salivary glands and mouthparts (including the stylet) of the pea aphid *Acyrthosiphon pisum*, providing a mechanism to transfer the symbiont into the host plants. *S. symbiotica* was also detected in plant tissues wounded by the penetrating stylet and was further transferred to naïve aphids feeding on plants infected with this symbiont. Proteomic analysis of the supernatant from *S. symbiotica* cultures in liquid medium revealed the presence of known and novel proteases including metalloproteases. The transcripts encoding these *S. symbiotica* enzymes were identified in both aphids and infected plants. The *S. symbiotica* enzymes may cooperate with aphid salivary enzymes injected into the plant, facilitating the digestion of phloem sap proteins and thereby supporting the suppression of plant defenses.

Highly efficient CRISPR Cas HDR for genome editing in insects

Roswitha A. Aumann[1], Irina Häcker[1,2], Marc F. Schetelig[1,2],

[1]*Institute for Insect Biotechnology, Department of Insect Biotechnology in Plant Protection, Justus-Liebig-University, Giessen, Germany*
[2]*Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Division of Bioresources, Department of Insect Pest and Vector Control, Giessen, Germany*

Corresponding author: roswitha.auermann@agrar.uni-giessen.de

The Mediterranean fruit fly, *Ceratitis capitata* (Medfly) is one of the most successful invasive insect pests causing vast economical damage in horticultural systems worldwide. A currently used strategy to control this pest insect is the sterile insect technique (SIT), an effective area-wide, environment friendly, and species-specific control method. SIT is based on the mass release of radiation-sterilized males that cause infertile matings with females in the field. Repeated releases result in population reduction. Despite being a very successful strategy, there are several key aspects of SIT that can be improved to increase the efficacy. One is the large-scale production of competitive male-only populations for the release, as male-only releases are more efficient than mixed releases. Such sexing strains could be generated by molecular genetics. CRISPR-Cas genome engineering to date is the most promising method to improve key aspects of the SIT like the creation of sexing strains. Moreover, compared to transgenic technologies, there is the possibility that certain CRISPR-induced mutations in organisms could be classified as a non-GMO in the US and Europe, thus avoiding GMO regulatory restrictions.

Here, we report the first successful CRISPR-Cas9 homology directed repair (HDR) genome editing in Medfly. By injecting pre-assembled Cas9 ribonucleoprotein complexes, loaded with two different guide RNAs, and a short single-stranded oligodeoxynucleotide repair template we induced the exchange of three base pairs and thereby converted the enhanced green fluorescent protein of a transgenic *C. capitata* strain into the blue fluorescent protein. Overall, six out of seven fertile and individually backcrossed G_0 individuals generated 57-90% knock-in rate within their total offspring. As this is also the first report of CRISPR-Cas9 HDR in the family of Tephritidae, it is an important step towards the application of this technique to other important Tephritid fruit pests like *Bactrocera dorsalis, B. oleae*, *Anastrepha ludens*, or *A. suspensa*, and will be crucial for the development of non-transgenic strategies to fight these pest insects.

Approaches towards heterologous expression of biosynthetic gene clusters for the production of natural products

Nils Böhringer, Till F. Schäberle

Institute for Insect Biotechnology, Justus Liebig University Giessen, Giessen, Germany

Corresponding author: Nils.Boehringer@bio.uni-giessen.de

Microorganisms, *e.g*. bacteria and filamentous fungi, associated with insects represent a most promising bioresource for specialised natural products, which are showing a vast spectrum of bioactivities. For many of these microorganisms knowledge about suitable cultivation conditions is missing and therefore, their metabolic properties are underinvestigated. Furthermore, this lack of knowledge goes hand in hand with the fact that tools for genetic manipulation are absent.

To overcome these limitations, heterologous expression of biosynthetic gene clusters (BGCs) coding for the biosynthesis of natural products in well investigated host organisms is a promising approach. In addition, it opens the door towards the analysis of BGCs from the so-called microbial dark matter, *i.e*. strains which are not culturable under laboratory conditions yet. Hence, by heterologous expression, genetic manipulation of novel biosynthetic pathways becomes feasible and the laborious and time consuming *de novo* development of genetic tools can be avoided. Modern molecular biological methods like Gibson-Assembly or transformation-assisted-recombination (TAR) in yeast facilitate and accelerate cloning and heterologous expression of large BGCs. Examples from the toolbox comprising most contemporary cloning methods, which was established in our laboratories, will be presented.

Harm and heal? Pharmacological potential of animal venoms

André Billion

Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources Giessen, Germany

Corresponding author: Andre.Billion@ime.fraunhofer.de

Drug resistances, neglected diseases, new molecular targets, and drug efficacy are major challenges of today's pharmaceutical industry. A highly promising natural source of potent bioactive compounds comprise animal venoms. Venoms are complex cocktails of up to 1000 toxins targeting all major physiological pathways. This high toxin diversity available in thousands of venomous species lead to a rough estimate of 20 million pharmacological valuable compounds across animal venoms. Their pharmacological potential is evident by the 18 toxin-based drugs currently on the market.

However, pharmacological assessment of unknown natural sources is highly time and cost consuming, and c. 90% of all drugs fail in clinical trials due to lack of efficacy. Therefore, the drug discovery workflow needs to be strengthened by combining traditional with modern techniques, such as genetics and high-throughput screenings, making a higher variety of data available for a more precise functional prediction. Applying the novel concept, the evaluation of animal venoms potential and selection of putative high potential candidates occurs stepwise by performing: i) Evolutionary studies to comprehend the framework in which the venom system evolved; ii) primary biochemical assessment by chromatography-bioassay to estimate the venom's potency and variability; iii) transcriptome analyses to draw the putative multi-level venom composition; and iv) high-content screening to characterize the venom's bioactivity.

This reinforced drug discovery workflow is supposed to improve the access to the high potential of animal venoms, while increasing the rate of success of drug candidates.

Development of insect pathogens as biological control

Tessa Carrau, Nils Hiebert, Merle Bartling, Kwang-Zin Lee

Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources, Giessen, Germany

Corresponding author: kwang-zin.lee@ime.fraunhofer.de

Invasive alien species are a major threat to human livelihoods and biodiversity according to the millennium ecosystem assessment from the United Nations. Biological control measures against arthropod invasive species are urgently needed to protect agriculture and biodiversity. Entomopathogens including insect-specific viruses, bacteria, fungi and nematodes offer a source for ecological safe control agents for the management of arthropod pests. Here we describe the identification and isolation of entomopathogens against the spotted-wing drosophila, *Drosophila suzukii*, a major invasive pest of ripening soft fruit. The local collection of moribund *Drosophila suzukii* larvae yielded promising entomopathogenic candidates, such as Drosophila-specific viruses as well as bacteria. The characterization of those candidates and further strategies for the development of control agents will be presented here.

Combined microfluidics/FACS platform for antibiotic drug discovery of insect-associated microorganisms

Markus Oberpaul[1,2], Marius Spohn[1,2], Stephan Brinkmann[1,2], Maria Patras[1,2], Jochen Fracowiak[1,2], R. Plarre[3], Dino-Peter McMahon[3], Peter Hammann[1,4], Andreas Vilcinskas[2], Jens Glaeser[1,2]

[1] *Sanofi-Fraunhofer Center of Natural Product Research, Giessen, Germany*
[2] *Branch for Bioresources of the Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany*
[3] *Bundesanstalt für Materialforschung und -prüfung, Berlin, Germany*
[4] *Sanofi-Aventis Deutschland GmbH, Industriepark Höchst, Frankfurt-Höchst, Germany*

Corresponding author: Markus.Oberpaul@ime.fraunhofer.de

Multi-resistant Gram-negative pathogens pose a major threat to public health worldwide. There is an urgent need for the development of novel approaches to discover new antibiotics. Our work focuses on the cultivation and screening of untapped bioresources via high-throughput approaches using a microfluidics- and flow cytometry-based platform, with emphasis on activity against Gram-negative bacteria.

The search for novel compounds is a numbers game. We implemented a microfluidic platform for the parallel cultivation of hundreds of single cells from an fresh environmental sample. Millions of agarose microspheres, so-called droplets, were used to encapsulate single cells from termite nest samples with GFP-tagged screening strains simultaneously. Following a short incubation period, droplets which showed no gain of fluorescence, potentially containing microcolonies originated from single cells of termite nest-associated microorganisms, were selected using fluorescence-activated cell sorting (FACS) and were distributed into microtiter plates. This cultivation and selection process was followed by rapid validation of activity in small volumes of liquid culture. The analysis of active fractions was carried out automatically using high-resolution UPLC-MS and compared against our in-house database. We were able to identify active compounds against *E. coli*, *S. aureus, C. albicans,* and *M. smegmatis*.

Our miniaturized, microfluidics- and FACS-based platform enables the cultivation of environmental cells and the subsequent bioactivity screening of millions of events on a microlitre-scale, in a streamlined and cost-effective manner. This opens up immense opportunities for finding novel bioactive compounds suitable for lead identification in the future.

Evolutionary venomics of neglected pancrustaceans

Björn M von Reumont

Institut für Insektenbiotechnologie, JLU Giessen, Department of Life Sciences, Natural History Museum, Giessen, Germany

Corresponding author: Bjoern.Reumont@ime.fraunhofer.de

Venoms are employed by species as evolutionary key adaptations for defense, predation or competition. Yet, for most taxa their biology and venoms are only vaguely known. Also, the processes that shape evolution remain generally understudied. Modern evolutionary venomics addresses as main question how venoms evolve by applying a plethora of –omics methods. These recently became so sensitive and enhanced that smaller, neglected pancrustaceans are now more easily accessible to comparatively study their venoms. More knowledge about these taxa is essential to better understand venom evolution in general, but also to harvest from an applied perspective a vast potential of new, bioactive compounds.

Robber flies (Diptera, Asilidae) were early suspected to be venomous due to their ability to overpower well-defended prey, for example dragonflies or hymenopterans. However, their venom remained unstudied. Utilizing all fields from evolutionary venomics we show that asilid venom glands secrete mostly venom peptides and non-enzymatic proteins. Many of them are unknown peptides, including a new knottin-like neurotoxin. The novelty of these peptides suggests that the robber fly venom system evolved independently from hematophagous dipterans and other insects. Genome data supports indeed a dynamic, multi-modal process of venom evolution in robber flies.

In contrast, the venom of *Xibalbanus tulumensis* – an underwater cave dwelling remipede crustacean - resembles a complex cocktail of peptides and enzymatic proteins. In total, 32 expressed and secreted venom protein families were identified via proteomic and transcriptomic analyses. These include 13 novel peptide classes, of which four lack similarities to any known structural class. Of particular interest are double-knottin structured peptides that are so far only known as neurotoxic components from spider venom.

Recombinant production of insect derived antimicrobial peptides in stably transformed *Drosophila melanogaster* S2 cells

Jan Zitzmann, Tobias Weidner, Peter Czermak

Technische Hochschule Mittelhessen - University of Applied Sciences,
Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany

Corresponding author: jan.zitzmann@lse.thm.de

Antimicrobial peptides (AMPs) from insects are valuable resources for pharmaceutical industry and can serve as leads for the development of novel antibiotics. To access this potential an effective recombinant expression process is mandatory, which includes the selection of a suitable expression host as well as process optimization during scale up. Here we describe the recombinant production of AMPs derived from the greater wax moth *Galleria mellonella* or the ladybug *Harmonia axyridis* using stably transformed *Drosophila melanogaster* S2 cells. Based on the polyclonal population that was obtained after transfection, we isolated highly productive single cell clones by limiting dilution and achieved a two to sixfold increase in productivity. Further optimization on the cellular level included a statistical planned screening to determine optimal conditions for induction of the employed Metallothionein promoter. The online measurement of the cell suspensions dielectric properties and turbidity enabled an efficient process control and monitoring of the cells physiological status. Based on this information, 25 mg of the AMP was expressed at the 1-L bioreactor scale in batch mode. Current focus of this work is the employment of tangential flow filtration to enable efficient perfusion processes. Using a short perfusion run at the bench scale already increased the final protein yield to 130 mg using essentially the same equipment as for batch culture. Finally, the functional AMPs were recovered by affinity chromatography and the antimicrobial properties against model strains of Escherichia coli were tested, indicating the successful isolation of active peptides.

Oral Presentations

Session 5 – 12

Topic 1:
Food safety for using insects

Topic 2:
Insect production systems

Topic 3:
Applications of insects as feed and food

Topic 4:
Non-food applications of insects

Microbial community dynamics and functions in Black Soldier Fly larvae (*Hermetia illucens*, Diptera: Stratiomyidae) reared on various diets

Thomas Klammsteiner[1], Andreas Walter[1], Carina Desirée Heussler[2], Blaž Stres[3], Florian M. Steiner[2], Birgit C. Schlick-Steiner[2], Wolfgang Arthofer[2], Heribert Insam[1]

[1] *University of Innsbruck, Department of Microbiology, Innsbruck, Austria*
[2] *University of Innsbruck, Department of Ecology, Innsbruck, Austria*
[3] *University of Ljubljana, Department of Animal Science, Domžale, Slovenia*

Corresponding author: thomasklammsteiner@hotmail.com

Investigating the influence of various diets on the composition of the gut microbiome is a frequently chosen approach to unveil the who's who in regards of substrate-degradation capabilities in an organism, regardless if human or insect. Our study shows that three separately tested substrates (chickenfeed, grass cuttings, fruit/vegetable mix) have a formative impact on the larval gut microbiome of the Black Soldier Fly (*Hermetia illucens*). However, it is likely that a stable and robust core microbiome represents the base for the growth and survival of larvae on a variety of diets. This core microbiome establishes during early developmental stages and is defined by only a handful of microorganisms that account for most of the sequences seen in metagenomic data, while an extensive number of less abundant microbes contribute to food-regulated variation in the gut communities. In our analysis, we applied various statistical measures to assess if above-mentioned diets significantly impact the composition of the gut microbiome over time and if the growth of substrate-specific bacterial indicator species is influenced by the type of food.

Transmisson of Salmonella sp. from contaminated wheat bran to mealworms (*Tenebrio molitor*)

Enya Wynants[1], Lotte Frooninckx[2], Sabine Van Miert[2], Johan Claes[1], Leen Van Campenhout[1]

[1] *KU Leuven, Microbial and Molecular Systems, Geel, Belgium*
[2] *Thomas More University College of Applied Sciences, RADIUS, Geel, Belgium*

Corresponding author: enya.wynants@kuleuven.be

Coleoptera species have been suggested to serve as a vector for Salmonella sp. in poultry houses (WALES *et al.* 2010). Furthermore, one DNA-based study (OSIMANI *et al.* 2017) indicated the presence of Salmonella sp. in a food including *Tenebrio molitor*. The transfer of Salmonella sp. from the rearing substrate to mealworms was never studied before. Therefore, in this study, wheat bran was inoculated with either 4 or 7 log cfu/g of Salmonella enterica in order to study its transmission to the larvae reared in the wheat bran. Mealworms were added to the inoculated substrate and kept in a climatic chamber (28°C, 65% RH). Samples of larvae and substrate were analysed for their number of Salmonella sp. after 1, 3 and 7 days after inoculation. The number of Salmonella sp. highly fluctuated between days during the sampling period. However, after 7 days, larvae and substrate in the test with an inoculation level of 7 log cfu/g remained contaminated with Salmonella sp. ranging from 2.6 to 5.2 log cfu/g. In the test with an inoculation level of 4 log cfu/g, however, Salmonella sp. counts were below the detection limit (1 log cfu/g) for all larval and most substrate samples. In contrast, Salmonella sp. could still be detected in the substrate without larvae (control) for both inoculation levels. These preliminary results indicate that contamination of Salmonella sp. at levels below 4 log cfu/g in wheat bran will likely not result into long-term colonisation of the mealworms, but further research is certainly necessary.

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Identification, heat-survival and outgrowth of endospore-forming bacteria harboured by edible insects

Dries Vandeweyer[1], An Borremans[1], Christel Verreth[2], Bart Lievens[2], Leen Van Campenhout[1]

[1] KU Leuven, Microbial and molecular systems Geel, Belgium
[2] KU Leuven, Microbial and molecular systems, Sint-Katelijne-Waver, Belgium

Corresponding author: dries.vandeweyer@kuleuven.be

Edible insects are increasingly being investigated for their microbial quality and food safety. Endospore-forming bacteria are reported to be present in edible insects in varying quantities. Bacterial endospores are known to easily survive processing (e.g. heating) steps applied on foods. Since this may involve a risk for food safety, in this study endospore-forming bacteria present on fresh edible mealworms and crickets were assessed and quantified using plate-counts, followed by 16S ribosomal RNA gene based identification. Additionally, heat-survival of both vegetative cells and spores after a short boiling step (40 s for mealworms, 90 s for crickets) as well as outgrowth of spores during refrigerated (4°C) storage up to 24 days were studied.

Results confirm the rapid reduction of vegetative bacteria after boiling. Endospores showed variation in heat-resistance, but generally survived easily. During refrigerated storage, bacteria slowly grew to reach the spoilage threshold of 7 log cfu/g after at least 21 days for mealworms. While the microbial load of crickets was generally higher than that of mealworms, the outgrowth after heat treatment was slower and did not reach the 7 log cfu/g threshold, even after 24 days of storage. Surviving endospores were able to germinate during the first days of the storage period and were found to sporulate again from day 18. From a microbiological point of view our results suggest that chilled storage of maximum 18 days after a heat treatment can be applied in order to maintain food safety of the insects.

Optimization of decontamination and drying processes to secure safety and storage stability of Black Soldier Fly products (*Hermetia illucens*)

M'ballou Cissé[1], Yolaine Leboeuf[1], Marie-Hélène Deschamps[1], Grant W. Vandenberg[1], Cristina Ratti[2], Linda Saucier[1]

[1] *Université Laval, Département de sciences animales, Québec, Canada*
[2] *Université Laval, Département des sols et de génie agroalimentaire, Faculté des sciences de l'agriculture et de l'alimentation, Québec, Canada*

Corresponding author: mballou.cisse.1@ulaval.ca

Whole insect larvae and insect meals are new alternative products to feed animals. Conventional processes such as boiling, and air-drying can reduce contamination and extend product shelf life. However, suboptimal processing may have deleterious effects on product quality. The purpose of this study was to characterize the effects of different decontamination (boiling) and preservation (hot air drying and freeze-drying) methods on the water content, microbial load and larval product quality. Black soldier fly larvae (*Hermetia illucens*) were produced in the *Laboratoire des sciences aquatiques et médicales* of Université Laval (Québec City, Canada). After being fed 10 days on a Gainesville reference diet (70% moisture) under controlled conditions (27°C), the larvae were harvested and rinsed with distilled water before being euthanized by freezing at - 40°C. Whole larvae were decontaminated by plunging in boiling water (40 sec, 2, 4, 6 and 8 min) or punctured before being dried to facilitate water loss before hot air drying (60ºC for 4, 6, 8, 10 and 14 hours) or freeze-drying (40°C for 14, 24, 48, 72, hours). The most effective process was determined by comparing drying curves (i.e., time to obtain a water content < 0.1 g water/g dry matter) and water activity of the product ≤ 0.3. The microbiological load of fresh, boiled and punctured larvae at optimal drying times was evaluated by counting total aerobic mesophilic (AMT), *Listeria* spp., presumptive lactic acid bacteria (LAB), presumptive *Staphylococcus aureus*, *Enterobacteriacea*, *E. coli* and coliforms. The impact of decontamination and preservation processes on quality product was assessed by colour (L * A * b * colour values), pH and lipid oxidation methods (TBARS, Xylenol Orange). Scalding at 100 °c / 8 min followed by hot air drying at 60°c / 4 hours decreases the microbial load of AMT, LAB and *E. coli* with a logarithmic reduction of up to, respectively, 4, 1, 4, 2 and 3, 9 log CFU/g comparatively to the initial load prior to processing.

Key words: Black soldier fly larvae, blanching, hot air drying, freeze-drying, larval quality.

The use of increasing false flax [*Camelina sativa* (L.) Crantz] cake levels as rearing substrate of Black Soldier Fly (*Hermetia illucens*) larvae

Marco Meneguz[1], Incoronata Galasso[2], Giovanna Battelli[3], Remo Reggiani[2], Francesco Gai[4], Laura Gasco[1]

[1] University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, Italy
[2] National Research Council, Institute of Agricultural Biology and Biotechnology, Milano, Italy
3 National Research Council, Institute of Science of Food Production, Milano, Italy
4 National Research Council, Institute of Science of Food Production, Grugliasco, Italy

Corresponding author: laura.gasco@unito.it

False flax (*Camelina sativa*, CS) is an oilseed crop used for the production of biofuels. Compared with other conventional oilseeds (such as canola and sunflower) CS has several agrotechnical benefits. Indeed, the cultivation of the crop is simple and environmentally friendly, the application of pesticides/herbicides is not needed (ZUBR 2003), the plant is adaptable to marginal soils, showing good productivity (VOLLMANN *et al.* 2007, MOSER 2010, MASELLA *et al*. 2014), and may be a suitable candidate for biofuel production in marginal environments. The by-product (cake) obtained after the seed oil extraction is high in protein (350-400 g kg^{-1}) and contains a residual of oil of 100-130 g kg^{-1}. The energetic value of CS cake (MJ kg^{-1} DM) is higher for ruminants (15.0) than for pigs (14.0) and poultry (8.00) (BOHME & FLACHOWSKY 2015). A potential disadvantage of using CS cake in livestock diets is the presence of the glucosinolates (GSL) (RUSSO & REGGIANI 2017). However, to overcome this disadvantage, there are CS lines with reduced GSL content. The residual oil, present in the seedcake, is characterized by approximatively 90% of unsaturated fatty acids (FA) and the n^{-3} accounts for about 37.0% of the total FA content. Black soldier fly (BSF) is a generalist species able to reduce large quantities of organic substrates and thus consider as an interesting solution for the waste management (MENEGUZ *et al.* 2018). Moreover, as during their growth BSF larvae accumulate high quantities of nutrients, they are evaluated for their potential to produce products such as protein meal or fat for livestock feeds.

The lipid content of BSF larvae is largely dependent on the substrate used as diets and on their stage of development (MENEGUZ *et al.* 2018). In particular, the FA profile of the diet dramatically influence the BSF larvae FA composition. Due to the valuable presence of n-3 FA in CS seed cake, the aim of the present work was to assess the impact of CS cake on the development of BSF larvae and to evaluate if it is possible to enrich the BSF larvae n-3 FA content. A trial was carried out at the Experimental Facility of the Department of Agricultural, Forest and Food Sciences (DISAFA; University of Torino, Torino, Italy). A standard crop diet (Gainesville diet) GA was used as control diet. Four experimental diets were formulated with a substitution of 25% (CS25), 50% (CS50), 75% (CS75) and 100% (CS100) of control diet with a CS low GSL cake content. Six

replicates per treatment were performed and the replicates were fed daily with 16 g of substrate on a wet basis (70% moisture). Trial was performed in a climatic chamber with 28°C T and 70% RH. Trial was stopped when larvae overpass the average weight of 100 mg. Mortality, ingested food ((provided food-residues/provided)*100), final biomass and time were recorded. No differences were highlighted between the different group considering mortality (ranged between 89.0±15.96 and 99.3±0.69%), ingested food (ranged between 37.1±3.55 and 59.1±10.95%) and final biomass (ranged between 19.6±3.35 and 27.4±3.73), while time (ranged between 8.2±0.07 and 9.1±0.07 d) highlighted differences between CS25 and the other diet (CS50, CS75, CS100 and GA). Moreover analysis on the larvae FA profile showed that CS diets modify their FA profile compared to GA diet.

Influence of temperature, humidity and moisture source on the early development of the Yellow mealworm (*Tenebrio molitor*)

Carl Coudron, David Deruytter, Jonas Claeys, Stefan Teerlinck

Inagro, Roeselare, Belgium

Corresponding author: jonas.claeys@inagro.be

For scientific experiments with mealworm the following conditions are considered optimal: a uniform temperature of 26°C with a relative humidity (RH) of 70% and daily addition of a moisture source like carrot. However, little is known about specific growth conditions for mealworm hatchlings and their needs during the first weeks. Nevertheless, in other production animals like broilers optimal growth is achieved when the temperature steadily decreases from 32°C to 20°C before slaughter. Personal contact with several large scale breeders indicates that they also alter their climate depending on the growth stage of mealworm. Moreover there seem to be major differences between breeders on when to start the supply of a moisture source. All these factors indicate that the growth rate can be accelerated significantly by determining size specific optimal conditions (like temperature, humidity and moisture source supply).

To assess the influence of different parameters a comparison is made with a standard breeding method at 26°C, 70% RH and supply of moisture starting at day 28 after ending oviposition. In contrast to most lab scale tests, the experimental design is at a semi-industrial scale where rearing trays are commonly 600x400 mm to account for potential heat production and density effects. Literature indicates that higher humidity (RH > 70%) is beneficial for growth in all stages of mealworm development as they can absorb atmospheric water. Furthermore a study suggests that optimal mealworm growth occurs at 31°C.

Beetles oviposit during 7 days on a wire mesh in wheat flour. Eggs are separated from the flour by sieving and can then be evenly distributed over the experimental trays (60x40 cm). Each tray contains approximately 20.000 eggs and are supplied with wheat bran as a rearing substrate. Afterwards the trays are allocated to one of the 4 climate controlled rooms. 4 climate conditions are maintained for a period of 4 weeks: 27°C at 60% relative humidity (RH), 27°C at 80% RH, 31°C at 60% RH and 31°C at 80% RH. In each climate controlled room different feeding patterns for moisture source will be evaluated: the starting moment when moisture source is supplied varies. One group is supplied with daily moisture source right after ending oviposition, each next group is supplied with moisture source with one week delay such that the optimal starting point can be determined at different climate conditions.

Initial results indicate that by nursing them at 80%, growth can be sped up, dehydration of eggs can be prevented and need for an external moisture source might be postponed. Young mealworm eat a minimal amount of the moisture source and the remain-

der may be a source for fungal infections. However starting the supply of moisture source soon after hatching, has a positive influence on the growth rate of mealworm. By determining the optimal humidity and the minimal amount of moisture source necessary where everything is consumed and growth rate is enhanced, lifecycle can be shortened.

Bio-conversion of different organic substrates into high protein and fat Black Soldier Fly larval biomass for feed and non-feed applications

Anton Gligorescu, Christian Fischer, Lars-Henrik Heckmann

Danish Technological Institut, Bioengineering and Environmental Technology, Aarhus, Denmark

Corresponding author: angl@dti.dk

For the past 6 years, Danish Technological Institut has been working, nationally and internationally, with insect production and bio-conversion of different waste streams into insect biomass, in the form of housefly (*Musca domestica*) larvae, mealworm (*Tenebrio molitor*) larvae and black soldier fly (*Hermetia illucens*) larvae (BSFL). The work conducted using BSFL is developed on the circular economy paradigm and is mainly focusing on bio-conversion of different organic waste streams and food-grad substrates into BSFL biomass used for feed or non-feed applications.

The presentation will show some of the results generated during the bio-conversion of different organic substrates (i.e. household waste, catering waste, beach cast, spent grains and chicken feed); including:

i) Characteristics of different substrates in terms of dry matter (DM), protein, fat and ash.

ii) Performance of BSFL on different substrates with focus on development, weight and survival. The substrates characteristics were found to affect the development, weight and survival of BSFL.

iii) Efficiency of the BSFL production reared on different substrates with focus on: larval biomass production, feed conversion efficiency and substrate reduction. BSFL production efficiency was found to vary with the quality of the substrate, leading to high variation in FCR ranging from 1.8-10.

iv) Characteristics of: 1) larval biomass reared on different substrates: protein, fat and of 2) the insect frass: ash and NPK profile. The larval characteristics were found to vary accordingly to the substrates.

Nutritional requirements of Black Soldier Fly larvae

Thomas Spranghers, Sharon Schillewaert, Filip Wouters

VIVES Hogeschool, Agro-and Biotechnology campus Roeselare, Roeselare, Belgium

Corresponding author: thomas.spranghers@vives.be

Larvae of the black soldier fly (BSF), *Hermetia illucens*, have potential to be reared on a wide spectrum of organic waste streams. However, in practice, most types of vegetal waste streams are lacking certain nutrients which are essential to guarantee an optimal growth. Therefore, mixtures of different waste streams could be applied to guarantee a sufficient larval biomass production on low value, and consequently low cost, substrates. In order to identify possible interesting mixtures, the nutritional requirements of BSF larvae should be better understood. In this study, the optimal crude protein content of a BSF diet was investigated. The reference for optimal growth was a chicken-feed/water mixture (30/70) containing 5.4% protein. Consequently, the performances of BSF larvae fed different iso-energetic mixtures containing 5.4%, 2.7%, 1.4% and 0.7% protein, were compared. From the first results, it could be deduced that 2.7% protein in a wet mixture is sufficient for BSF rearing. Doubling that amount did not enhance the performance any more whereas lower levels of protein resulted in a reduction of harvested larval biomass. These findings are quite interesting given that most waste streams from vegetal origin contain substantially less crude protein than the chicken-feed/water standard. Moreover, the lower the amounts of crude protein in the BSF rearing diet, the fewer nitrogen could be emitted to the environment as ammonia.

Impact of beetle age and density on fecundity of *Tenebrio molitor*

Ida Elisabeth Berggreen, Jonas Lembcke Andersen, Lars-Henrik Heckmann

Danish Technological Institute, Life Science, Aarhus C, Denmark

Corresponding author: ideb@teknologisk.dk

To maintain a considerable larvae production, it is important to increase our knowledge on the reproduction of adult beetles. Industrial production of insects is more intense than that of wild populations, and the production of insects can be more effective, by investigating different parameters of beetle biology in their rearing environment.

In this presentation, we will show some of the results, that we have generated at the Danish Technological Institute (DTI), during recent and ongoing R&D projects. The results regard the effect of age and density of *Tenebrio molitor* beetles on reproduction. Some of the results have been presented in a newly published article (BERGGREEN *et al.* 2018), and will be discussed in relation to more resent data from a pilot scale production. In the article, four densities of beetles were used (0.11, 0.21, 0.42 and 0.84 beetles/cm^2). Densities from the pilot production varies more than in the controlled experiment, but has been divided into four groups (average of 0.31, 0.52, 0.73 and 0.98 beetles/cm^2). The number of eggs in a production tray is affected by both density and age of the beetles. However, in a production it can be favorable to use the density that results in the highest total output per tray, even though, it may not be the same density, that gives a maximum of eggs per female beetle. The presentation will discuss the similarities and differences between the experiment and pilot production.

Insects as feed for and from aquaponics: What about environmental issues?

Denise Ott[1], Jens Liebscher[2], Ariane Müller[3], Janine Dinske[4], Sebastian Braun[1], Dirk Schmidt[1], Jens Dautz[4], Herwig O. Gutzeit[3]

[1] *EurA AG, Erfurt, Germany*
[2] *Bio.S Biogas GmbH, Grimma OT Beiersdorf, Germany*
[3] *Technical University of Dresden (Institute of Zoology), Dresden, Germany*
[4] *TERRA URBANA GmbH Zossen, Germany*

Corresponding author: denise.ott@eura-ag.de

The world population and thus the demand for food will increase drastically in the coming decades. Fish and meat production and processing will grow proportionally. The significance of the effects of agricultural activities on water and land use, climate change and environmental degradation, such as eutrophication or terrestrial and marine acidification, is well acknowledged and has been demonstrated by many studies. In this context, within the last years insects are repeatedly discussed as a future-oriented, sustainable source of food, as the ecological, economic, physiological and ethical advantages outweigh those of meat.

In aquaculture systems, insects are also gaining increasing interest as feed to provide a sustainable alternative to the fishmeal paradox, whose production leads to a high consumption of resources and negative environmental impacts. Reducing the proportion of fish protein in favour of insect proteins in combination with vegetable feed components could significantly reduce environmental burdens.

But, to evaluate the ecological sustainability of insects for feed and food, the production process, and further, the whole life cycle chain needs to be considered. The methodology of Life Cycle Assessment (LCA) offers a well-established and standardized approach to quantify environmental impacts throughout the life cycle of a product. LCA has gained increased acceptance in different industrial sectors, e.g. food, chemical or automobile industry. So far, however, there are hardly any life cycle assessment studies that confirm the ecological sustainability of the production, processing, use and waste disposals of insects as feed in aquacultures or beyond. Herein, a review of current literature LCA studies dealing with insects for food and feed is given to outline bottlenecks and challenges to deal with. Further, our LCA activities within a research project are presented. Therein, the production of fish feed from *Hermetia Illucens* larvae and Lemna in an inline recirculating system is investigated. The research investigations are accompanied by LCA in order to quantify and objectively compare environmental impacts and to pave the way for sustainable alternatives for insect production systems and their application.

Comparison of wet and dry processing and fractionation for mealworms (*Tenebrio molitor*)

Verena Böschen

Research Institute of Feed Technology of the International Research Association of Feed Technology e.V. (IFF), Braunschweig-Thune, Germany

Corresponding author: iff@iff-braunschweig.de

Insects are in the focus of feed and food production as sustainable protein sources. With respect to the industrial scale production [1, 2], the yellow mealworm is a suitable option.

Pelleting is an important process in feed industry and more and more in aquaculture industry, too. A lot of parameters influence the pelleting process, e.g. raw material composition, meaning raw fibre, raw protein, fat or starch content, as well as the kind of starch or technological parameters like pellet diameter, the length of the press channel or the gap between roller and die.

At present, little is known about the processing features or the pellet quality of feed formulations including insect meal. Such information is crucial for adaption of techniques with respect to similar pellet quality with changing formulation.

Therefore, the aim of the investigations is to compare the impact of increasing content of in-sect meal on pellet characteristics without changing nutrition properties of the whole formulation.

The different characteristics of these pellets for fish and pork feed are presented.

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Marketing edible insects to vegans and vegetarians based on nutritional and ethical considerations

Josh Galt[1,2], Curran Hendry[1,2]

[1] *Micro Livestock International, USA*
[2] *Angka Changrit Kampuchea NGO, Phnom Penh, Cambodia*

Corresponding author: josh@entopian.com

This presentation will take a deeper look at the growing trend of vegans and vegetarians who are embracing Entomophagy, and adding insects to an otherwise plant-based diet. It will look at how insects can greatly enhance the nutritional intake of plant eaters, and methods to creatively convince them that eating insect products is desirable. Supporting evidence also examines the science behind insects not feeling pain, along with benefits of improved global sustainability that will come from eating insects vs other plant protein sources. Perhaps most crucially, we examine the ethical basis for vegans and vegetarians to accept eating insects in conjunction with their do-no-harm ethos. The presentation culminates in readily applicable strategies to optimize your brand's marketing for one of the fastest growing segments of the food industry, giving you useful messaging tools which can be immediately applied to attract the attention, sales, and loyalty from the whole-food, plant-based demographic.

Pharmaceutical potentials of insect larvae: a high-throughput proteomics and lipidomics approach

Vahideh Rabani, Hamed Cheatsazan, Siamak Davani

UFR de sciences médicales et pharmaceutiques, Université de Bourgogne Franche-Comté, Besançon, France

Corresponding author: vahideh.rabani@univ-fcomte.fr

Although collecting or farming insects has a very long history in some parts of the world, the idea of using food and farm waste to grow commercial quantities of edible, native and non-nuisance insects is a whole new field. Due to their great food-to-biomass transformation capacity, their miniature size and their short lifecycle, flies are thought to be the ideal candidates to simultaneously alleviate several environmental and economic issues. As such are growing global protein demand, negative economical consequences of global warming, and the huge amounts of plastic and bio-waste disposal. Nevertheless, fly farming and its downstream industries need to pass through several cultural, legal and technical barriers which efficiently reduces their rivalling capacity against the conventional unsustainable food sources. On the other hand, farmed fly species have usually been considered as a new food source and the majority of current literature is concentrated on their application in the food industry. Therefore, their potential applications in high added-value industries such as cosmetics and pharmaceutics have rarely been explored. Using high-throughput proteomics and lipidomics tools, we have constructed whole-proteome and whole-lipidome databases for the larvae of black soldier fly (*Hermetia Illucen*) and blowfly (*Lucilia* sp.). Using these databases we explore proteins and lipids with potential pharmaceutical and/or cosmetics applications. We then compared these data with proteomics and lipidomics of larval hydrolysates processed by a new technique. Finally, we discuss some other potential applications of processed and unprocessed fly larvae and the most important issues for high throughput studying of insects.

A new approach to control pest thrips (*Thysanoptera*) using artificial diets to test effects and impacts of active substances

Marcus Jilge, Stephanie Krueger, Julia Chuttke, Gerald Moritz

Martin-Luther-University, Developmental Biology, Halle/Saale, Germany

Corresponding author: marcus.jilge@zoologie.uni-halle.de

A few thrips species are pests of ornamental and agricultural plants and can cause high economic losses by feeding with their piercing and sucking mouth parts, especially if they are vectors of tospoviruses. To control these pest species the use of insecticides is widespread, giving rise to new resistances continuously. Hence, as a new approach we used various methods to apply different artificial diets to the most economically important pest thrips species of the world, Frankliniella occidentalis (Western flower thrips). Thereby, one diet allows the development from 24 h old 1st instar larvae to adult stages. Additionally, to observe individual survival probability and developmental time, a method was established to apply the diet to separated single larval instars. Applying the artificial diet to thrips permanently lead to a significantly longer developmental time (14d; control: 9.3d). In addition, to test an active substance we used neem-tree-bark-extract added to the successful diet in three concentrations (0.1%, 1%, 10%). The lowest and middle concentrations allowed thrips to develop to adults with a significant extended developmental time (0.1%: 16.3d, 1%: 15d), for the highest concentration (10%) no larval development has been observed. These methods provide the possibility to qualitatively and quantitatively test active substances (insecticides, antibiotics) and biological agents (bacteria, viruses) and thus may lead to new strategies of thrips pest control.

Purification of insect cell culture derived recombinant baculovirus by steric exclusion chromatography for cell therapy application

Keven Lothert[1], Felix Beyer[1], Gundula Sprick[1], Peter Czermak[1,2,3,4], Michael W. Wolff[1,5]

[1] *University of Applied Sciences Mittelhessen, Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany*
[2] *Kansas State University, Department of Chemical Engineering, Manhattan, USA*
[3] *Justus-Liebig-University of Giessen, Giessen, Germany*
[4] *Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources, Giessen, Germany*
[5] *Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Magdeburg, Germany*

Corresponding author: keven.lothert@lse.thm.de

For the purification of proteins and viruses a novel method based on membrane steric exclusion chromatography has proven to be an excellent alternative to common chromatographic applications. An important benefit of the technique is the fast and simple procedure at mild chromatography conditions as no harsh binding and elution buffers are needed. Moreover, the method is highly suitable as a platform technology since a selective retention of the product depends, among others, mainly on its size. The sample is initially mixed with a polyethylene glycol (PEG) containing buffer of choice (e.g. PBS or TRIS). As a result, the steric exclusion of a macromolecule (i. e. virus particle) from the polyethylene glycol and the stationary phase allows retention of the macromolecules based on their molecular weight and PEG-concentration. Carefully adjusting the polymer composition in the buffer, smaller process contaminants, i.e. host cell proteins and DNA, can be washed out, in contrast to the targeted larger virus particles. These are subsequently eluted reducing the PEG concentration in the mobile phase.

We describe the application of regenerated cellulose membranes as stationary phase to purify vesicular stomatitis virus glycoprotein (VSV-G) pseudotyped autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) baculoviruses derived from Spodoptera frugiperda cells (Sf9 cells) by SXC. The purified virus particles are used as gene transfer tools for human mesenchymal stroma cells. For this purpose, the virus harvest was clarified prior to the SXC by sequential centrifugation, starting from 250 xg up to 4700 xg). A design of experiment approach considering the PEG molecular weight, its concentration and the ionic strength of the elution buffer as critical process parameters was used in order to maximize virus yield and contaminant depletion. Within the design space, virus recovery was ≥70%. Without further nuclease treatment the depletion of double-stranded DNA was >90% and host cell proteins were reduced by >99% in the virus fraction. In conclusion, SXC can drastically reduce the process development in terms of time and equipment requirements for the purification of insect cell culture derived recombinant baculoviruses, as well as for the achieved purity which is superior over classical methods.

Crop protection – The global problem

Stuart Reynolds

Department of Biology and Biochemistry, University of Bath, Bath, UK

Corresponding author: S.E.Reynolds@bath.ac.uk

How will the growing human population of the world be fed in 2050? Crop breeding for increased yield, boosting fertiliser use and irrigation are all subject to diminishing gains, but reducing pre- and post-harvest crop losses offers an opportunity to boost production. Estimating global crop losses due to insect pests is difficult, but broad-brush estimates are possible. An important conclusion is that although existing crop protection practices are not very effective on a global scale, improving them can make a contribution but will not be enough alone. Future crop protection will be most challenging in sub-Saharan Africa because this is where extra food will be most required; it is also where existing crop protection efforts are least effective, and where poor farmers are least able to invest in crop protection. Current methods are not only expensive in themselves but also require infrastructure not available where it is needed. They also result in collateral damage to biodiversity and ecosystem services and may damage farmers' and consumers' health. Further, pesticide resistance dramatically limits the lifetime of useful crop-protection techniques including both chemicals and GM crops. Many problems arise when crop protection is used prophylactically. "Smart" crop protection techniques that deploy pesticidal defences only when the crop is attacked may alleviate all these problems. It's evident that (i) more effective, environment-friendly crop protection methods must be found; (ii) these technologies must be effective and easy to apply in low-tech agricultural environments; (iii) greater investment in crop protection research and education are urgently required.

Different rearing substrates exhibit direct influence on growth and macronutrient composition of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae at an industrial scale

Andrea Scala[1], Rosanna Salvia[1], Jeffery Tomberlin[2], Patrizia Falabella[1]

[1] *University of Basilicata, Department of Science, Potenza, Italy*
[2] *Texas A&M University, Department of Entomology, College Station, USA*

Corresponding author: patrizia.falabella@unibas.it

Black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), during the larval stage is able to feed on varied organic materials. This feature can be exploited for the application of innovative strategies for waste management at an industrial scale. The ability to accumulate high levels of proteins and lipids, allows the use of resulting larvae as animal feed or in technical applications. Approximately 10,000 larvae were reared on 7.0 kg of six substrates: apple, banana, spent grain from brewery waste, apple/banana, apple/spent grain, banana/spent grain. The effect of the diets on insect growth, larval yield, proteins and lipids content, substrate reduction was evaluated. Substrates' pH value variations during the experiment were measured. High larval growth was observed in all the selected diets with significant differences among larval yield. The highest growth rate was measured in larvae fed on spent grain and banana/spent grain mix. The highest percentage of crude proteins was measured in larvae fed on apples/spent grain mix, while the highest percentage of lipids was found in larvae fed on apple and on apple/banana mix. The variation of the pH value was dependent from the substrate. This study shows that waste management through *H. illucens* represents a new economic resource and opens new perspective for a sustainable environmental friendly industrial development. Results indicate that diet influence larval performances, yield and content of macronutrients. Single diets or mix can be selected to produce insects with the desired nutrient profile to satisfy different needs and to address the product to different markets.

Inhibition of histone acetylation and DNA methylation negatively affects life history traits of the pea aphid (*Acyrthosiphon pisum*)

Phillipp Kirfel[1], Marisa Skaljac[1], Jens Grotmann[1], Katja Michaelis[1], Andreas Vilcinskas[2]

[1] *Fraunhofer IME, Giessen, Germany*
[2] *Justus Liebig Universität Giessen, Insektenbiotechnologie, Giessen, Germany*

Corresponding author: phillipp.kirfel@ime.fraunhofer.de

Epigenetic mechanisms such as acetylation of histones and methylation of DNA play important roles in regulating gene expression in eukaryotes. These mechanisms are regulated by complex interplay among different enzymes. DNA methyltransferases (DNMTs) are mainly responsible for DNA methylation, whereas histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate histone modification. The acetylation of histones by HATs can increase accessibility of DNA to transcriptional factors and promote gene expression, whereas deacetylation process mediated by HDACs has the opposing activity. Inhibition of evolutionary conserved epigenetic mechanisms using specific inhibitors can negatively affect a range of life history traits of an organism. We investigated the fitness of harmful agricultural pest and model pea aphid (*Acyrthosiphon pisum* Harris) after chemically altering transcriptional activity of genes encoding components of HATs, HDACs and DNMTs. Specific inhibitors of both epigenetic mechanisms negatively affected A. pisum including its survival, reproduction and development. Remarkably, HDAC and HAT inhibitors severely compromised development of *A. pisum* with opposing effect on body weight. Observed fitness costs probably reflect significant changes in expression of several genes encoding HDACs and HATs after exposure to epigenetic inhibitors. Given that resistance to chemical insecticides develops rapidly in aphids, our study suggests that epigenetic apparatus holds the potential for discoveries of targets for novel insecticides or insect resistant crops.

Safe and sustainable feed for *Tenebrio molitor* from domestic side streams of bio-economy

Elina Hirvisalo[1], Maija Karhapää[1], Maarit Mäki[1], Pertti Marnila[1], Lindqvist Bengt[1], Hilkka Siljander-Rasi[1], Miika Tapio[1], Lauri Jauhiainen[1], Taina Jalava[1], Heikki Roininen[2], Jaana Sorjonen[2]

[1] *National Resources Institute Finnland, Helsinki, Finland*
[2] *University of Eastern Finland, Joensuu, Finland*

Corresponding author: elina.hirvisalo@helsinki.fi

The choice of feed constitutes forms a significant part of the sustainability of the production of insects. Omnivorous *Tenebrio molitor* has potential to be fed with side streams of bio-economy in industrial production, which would improve circular economy.

In our HyväRehu-project we investigated the growth of *T. molitor* on side streams of food, beer and alcohol industry. The aim of the study was to find domestic side streams that can substitute imported soya bean and domestic grain, which could be used directly in food production. In this presentation the results of analyses of the larvae and their feed are shown. We investigated the usability of protein-containing raw materials (fishmeal, cold pressed rapeseed and faba bean) and other side streams (potato protein, barley feed, beer mash, dried pea and carrot meal) as feed ingredients as mixture with inactivated brewery yeast and conventional feed ingredients. In each experiment 0.35 larvae cm^{-3} of approximately same size (initial size range was 5 – 12 mm) were grown in plastic containers for four weeks under controlled conditions (at 24.2 ± 0.2°C, about 60.3 ± 1.4% relative humidity). Fresh carrots and potatoes were included to provide source of water. After four weeks the growth and viability were measured. Five trials were established by a row-column experimental design. Used statistical model was based on the experimental design, taking into account variation between trials and using starting weight as a covariate. Growth and viability were assumed to be normally and binomially distributed, respectively. Differences in growth and viability were detected and the results confirmed the feasibility of the investigated side streams as feed ingredients for *T. molitor*.

Feed conversion efficiency and nutritional values of common house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*) fed on agro-byproducts

Mary Orinda[1], Reuben Mosi[2], Monica Ayieko[2], Fred Amimo[3], Hamisi Nchimbi[4]

[1] *Jaramogi Oginga Odinga University of Science and Technology, Department of Agricultural Economics and Agribusiness Management, Bondo, Kenya*
[2] *Jaramogi Oginga Odinga University of Science and Technology, Department of Plant, Animal and Food Sciences, Bondo, Kenya*
[3] *Jaramogi Oginga Odinga University of Science and Technology, Department of Health Sciences, Bondo, Kenya*
[4] *University of Dodoma, Department of Biological Sciences, Dodoma, Tanzaniz*

Corresponding author: maryakinyi2010@gmail.com

Mass rearing of crickets as a cheap source of protein, requires a cheap and sustainable source of feed to ensure sustainability in production and agro byproducts provide a likely promising source. Feeding crickets on agro by-products might alter their body composition and nutrient contents due to the different nutrient composition of the agro by-products. This study sought to evaluate the feed efficiency and nutritional components of the different cricket cohorts fed on different agro by product diets. Both *A. domesticus* and *G. bimaculatus* were fed on four diets: Poultry grower's mash as the control, Rice bran+ brewer's spent yeast (RBSY), Rice bran + Bloodmeal (RBBM) and Rice bran + brewer's spent grain (RBSG). Effects of dietary composition on feed conversion efficiency and proximate values were assessed. Diets affected cricket feed conversion efficiency such that experimental diets appeared to be favourable in terms of protein content but poor in terms of feed conversion efficiency. Feed conversion ratios of 1.6, 2.4, 2.3, 2.4 were recorded for *G. bimaculatus* and 2.6, 5.1, 5.8, 5.5 were recorded in *A. domesticus* for GM, RBSY, RBBM and RBSG diets respectively. Slight variability was observed regarding dry matter (DM), (92-95.99%), ASH (3.27-4.74%) and P (1.09-1.41%), however, substantial variability was recorded in crude protein (57.49- 71.09%) and crude fat (14.93-33.44%) between the two species. These variations assert the dietary effects on the body composition of the crickets. There is need to establish their digestibility to permit informed comparison with the conventional feed.

Key Words: Feed conversion efficiency, cricket, nutritional values

Exploring RNA viruses from edible insects: A case study using termites

Dino-Peter McMahon, Hannah E. Davis

Freie Universitat Berlin / Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin, Germany

Corresponding author: dino-peter.mcmahon@bam.de

Our understanding of RNA viruses from edible insects is minimal at best, with studies largely focusing on model insect species and those associated with obvious signs of disease. This represents a considerable gap in understanding, given the growing role of insects as a source of food and feed, as well as the more general relevance of insects in agriculture and health. Illness due to entomophagy is rare but well documented, including fatal cases following the consumption of termites. Termites are eaten commonly in tropical Asia, Africa and South America, and are among the insects with the highest recorded fat content. There are many species of termites, with a wide range of diets and habitats centering around the consumption of wood and soil substrates. In this study, we report the results from a survey of more than 30 cockroach and termite transcriptomes, with the aim of understanding the diversity and evolution of RNA viruses as well as other potentially pathogenic organisms that

are associated with this relevant but somewhat overlooked group of insects. We discuss our results in the context of the possible zoonotic risk posed by insects, as well as in the context of emerging viral and other disease threats that may face insects being reared at industrial scales.

An optimized feeding strategy for Black Soldier Fly larvae (*Hermetia illucens*, L) for biomass production and organic waste reduction

Evans Nyakeri

Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

Corresponding author: evans.nyakeri@gmail.com

Urban centres of developing countries are characterized by poverty, informal settlements and non-collection of organic wastes. The later is primarily due to lack of economic incentives for those involved. A valorization technology using larve of the Black Soldier Fly transforms organic waste into a nutritious and valuable biomass for economically sustainable disposal of organic wastes. This study aimed to establish a feeding strategy for production of a Kenyan strain of BSF on feacal sludge (FS) supplemented with local organic substrates. Larvae feeding experiment were used to investigate effects of feeding rates, regimes and substrate combination and data for larval growth, bioconversion efficiency and prepupa nutrient content was collected. Biomass yield, substrate reduction and maturation days at feed rates of 100, 150, 200 and 250mg/l/d were 124g ± 3.0, 81% ± 8.9, 16; 140 ± 1.3, 84% ± 0.3, 16; 176 ± 3.3, 57% ± 1.1, 17 and 190g ± 1.2, 54% ± 1.2, 20 respectively. Daily feeding regime produced higher biomass yields, substrate reduction and shorter maturation period (201g ± 7.4, 84.6% ± 0.2; 16 days) compared to lump-sum feeding (204g ± 2.1; 77.1% ± 0.1; 20 days). When combined at ratios of 30:70, 50:50 and 70:30, all the parameters significantly improved, with the best compromise ratio for biomass yield and substrate reduction being obtained at 50:50 level. The study recommends daily feeding of a diet made up of FS and the co-substrates at a ratio of 1:1 and rate of 150-200mgl/d as the optimal strategy for waste management and biomass production.

Key words: Black soldier fly, larvae, prepupa, bioconversion, waste reduction, biomass

Modular insect plants – a crucial concept to penetrate the animal feed market

Paul Antony Whittle

Buhler Insect Technology Solutions AG, Plot B7-B, Wuxi National Hi-Tech Industrial Development Zone Xuedian Road, Wuxi, China

Corresponding author: paul.whittle@buhlergroup.com

Insects are part of the natural diet of many farmed animals including fish, chicken and pigs. Hence, many insect producers target the animal feed market due to the high acceptance of insect-based products by the animals. However, feed producers evaluate their ingredients according to parameters such as price, quantity, quality, consistency, and reliability of supply, which makes it difficult for insect-based products to penetrate the market quickly. Especially the demonstration of the benefits of the new ingredients is critical, because it helps to attract attention of potential feed producers and to identify promising business cases. These considerations make it difficult for insect producers to decide about the ideal start-up production capacity. Large facilities are favorable due to economy of scales, while smaller facilities will enable the development of the application market and minimize the investment risk. A promising solution to cope with this dilemma are modular and scalable insect plants.

A modular design allows to envision the final large scale facility, but building it in several phases. A key element of this concept are standardized rearing units, in which the growth of the insects is taking place. The number of rearing units determines the total amount of feedstock to be converted into larvae biomass. The integration of such standardized units makes the design of insect plants very flexible and the capacity of the plant can be easily adapted to feedstock availability or products needed. Modular design is also important in that capacity expansions can happen quickly due to efficient installation and start-up procedures. Traditionally different processing machines are shipped individually to the production site, where it is installed incrementally. Nowadays it is more common to work with modular process skids. Skids are process units containing several equipment that is assembled within a frame. This concept brings several advantages as the skids are typically assembled offsite under controlled conditions without affecting ongoing operations in the plant. Skids can be also tested before shipping to the site, which reduces on-site start-up time. In addition, the compact design allows an easy transport of the system and facilitates the rapid integration into existing plants.

In this presentation the opportunities of modularization in designing insect plants are shown. A plant to rear and process black soldier fly larvae will be used as a reference. Besides the phased approach for a gradual plant expansion, also the benefits of pre-assembled skids are discussed in detail. Finally, the presentation will highlight how modular plant design can help insect producers to meet the criteria of the animal feed industry, while limiting their initial capital investment for starting up an industrial production.

Insects as a source of vitamin B12 – Opportunities and obstacles

Anatol Schmidt, Helmut K. Mayer

BOKU – University of Natural Resources and Life Sciences Vienna, Department of Food Science and Technology, Food Chemistry Laboratory, Vienna, Austria

Corresponding author: anatol.schmidt@boku.ac.at

Insects are widely considered as highly nutritious, not only as a supplier for major nutrients such as protein and fat, but also for minor nutrients (e.g., vitamins). Vitamin B12 is of utmost importance for a balanced diet. It is especially difficult for vegetarians and vegans to meet their needs of vitamin B12, as it occurs almost exclusively in food of animal origin. Insects are considered to be rich in vitamin B12, which unfortunately is supported by very little scientific evidence.

The proposed work will introduce the highly complex and exciting biochemistry of vitamin B12 and its different forms, also known as vitamers. Various methods for the determination of vitamin B12 have been proposed (microbiological, immunological, chromatographic), each with distinct advantages and drawbacks.

Based on a combined approach utilizing immunoaffinity and ultra-high performance liquid chromatography (UHPLC), a validated study on the distribution and content of vitamin B12 in four edible insect species will be presented. Although the results confirm that whole insects (mealworm, grasshopper, cricket and cockroach) are high in vitamin B12, the presence of the biological none-active Pseudovitamin B12 must be considered. This raises serious questions regarding previously reported values for vitamin B12 in insects. Furthermore, the acquired knowledge should be considered regarding rearing-conditions as well as processing and extraction of major nutrients from insects.

Comparison of suitable drying techniques on nutritional quality of mealworms (*Tenebrio molitor*)

Nina Kröncke[1], Verena Böschen[2], Claudia Keil[3], Sandra Grebenteuch[4], Sebastian Demtröder[1], Andreas Thünemann[5], Lothar Kroh[3], Hajo Haase[3], Rainer Benning[1]

[1] University of Applied Sciences Bremerhaven, Institute of Food Technology and Bioprocess Engineering, Bremerhaven, Germany
[2] Research Institute of Feed Technology of the International Research Association of Feed Technology e.V., Braunschweig-Thune, Germany
[3] TU Berlin, Institute of Food Technology and Food Chemistry, Department Food Chemistry and Toxicology, Berlin, Germany
[4] TU Berlin, Institute of Food Technology and Food Chemistry, Department, Food Chemistry and Analytics, Berlin, Germany
[5] Federal Institute for Materials Research and Testing (BAM), Berlin, Germany

Corresponding author: nkroencke@hs-bremerhaven.de

The yellow mealworm (*Tenebrio molitor* L., *Coleoptera*: *Tenebrionidae*) is an edible insect, due to its ubiquitous occurrence it is well suited for an industrial-scale production (EFSA 2015, VAN HUIS 2013). Drying is an important preservation step in industrial insect production and processing. Within this study the effects of different drying technologies (freeze drying, microwave drying, rack oven drying, vacuum oven drying, fluidized bed drying) and process parameters on the fatty acid composition, lipid peroxidation and the appearance of volatile compounds in *Tenebrio molitor* larvae were investigated. Moreover, larvae were analyzed for total zinc values as well as the bioaccessibility of this essential mineral upon in vitro digestion.

Currently, the information about the stability of functional and nutritional components in mealworms during drying is still limited. Deepening the knowledge of process-induced changes of mealworm`s quality is urgently needed to improve drying techniques, and therefore to reduce undesirable properties of *Tenebrio molitor* while maintaining the required nutritional characteristics.

Product development of food with edible insects

Simon Hvid[1], Thomas Petersen[1], Lars-Henrik Lau Heckmann[2], Anne Louise Dannesboe Nielsen[1]

[1] *Danish Technological Institute, Food Technology, Aarhus, Denmark*
[2] *Danish Technological Institute, Water and Resources, Aarhus, Denmark*

Corresponding author: thpe@teknologisk.dk

The work focuses on integrating edible insects into food, by investigating insect components as ingredients while also looking at how to develop food products with main emphasis on application in meals.

There are many edible insect products available in grocery stores around Europe and the world. The vast number of these are in the snacks or mini-meal segment like bars, juices, etc. To really allow insects to feed the world and make a difference in the sustainability agenda, there is a need to make a transition from snacks into meals or meal components.

Several applications like bread, beverages and spreads have been tested in ongoing R&D projects - e.g. the in VALUABLE project – to implement the flavors and properties into meal products. The main outcome of these projects will be presented here.

To ensure the development of high-quality foods, the studies undertaken have included analyses of the insect components as functional ingredients, including:

- Functionality testing of defatted insect protein – emulsifier, foaming agent, water and lipid binding
- Investigation of fat extraction techniques
- Analysis of fat composition
- Possible applications for the fat fractions
- Application testing of protein fraction (and nonprocessed meal) – bread, beverages, spreads, snacks

Functionality and application of proteins will be presented, as well as the extraction, fractionation and application of lipids. The future offers further opportunities in all these areas, as well as sensoric comparison of insects. This will be discussed here as well.

Bees meal and cuticle-poor Black Soldier Fly meal, used as a sole source of protein, show casein-matching body weight and body protein gain as well as casein-matching body protein retention efficiency in C57BL/6 mice

Roee Gutman[1,2], Matan Fialko[1,3], Jean-Jacques Itzhak Martinez[2,4], Adi Jonas-Levi[5]

[1] *Laboratory of Integrative Physiology (LIP), MIGAL - Galilee Research Center, Kiryat Shmona, Israel*
[2] *Department of Animal Sciences, Faculty of Sciences and Technology, Tel-Hai Academic College, Upper Galilee, Israel*
[3] *Department of Biotechnology, Faculty of Sciences and Technology, Tel-Hai College, Upper Galilee, Israel*
[4] *Laboratory of Animal Ecology and Biodiversity, MIGAL - Galilee Research Center, Kiryat Shmona, Israel*
[5] *Department of Food Sciences, Faculty of Sciences and Technology, Tel-Hai Academic College, Upper Galilee, Israel*

Corresponding author: roeeg@migal.org.il

Little is known about the ability of various types of edible insects to support muscle growth in monogastric animals. Additionally, the high level of cuticle in insect meal may have negative effects on the digestibility and other characteristics of insects' protein quality. We herein tested the effect of feeding C57BL6/J male mice with two types of known edible insects: *Hermetia illucens* (Black Soldier Fly larvae – BSF) and *Apis mellifera* (Western Honey Bee larvae) as the sole source of dietary protein, on body weight growth curve, energy intake and energy digestibility, nitrogen true digestibility and body protein gain. More importantly, we tested the effect of feeding mice with protein rich, yet cuticle poor fractions, extracted from BSF and Bee meals. All insect-based diets were izonitrogenous and their effects were compared with that of feeding on the regular chow diet and an izonitrogenous casein-based diet. Our results show that weight gain of mice fed with BSF-meal (BSF) match that of mice fed on regular chow diet (Chow) yet is slower compared to that of mice fed on casein-based diet (Casein). However, that was not the case for the body weight gain curve of mice fed on Bees-meal (Bees) that matched that of the Casein. Feeding mice with izonitrogenous BSF-meal, yet with lower levels of cuticle (BSF-1), resulted in a faster weight gain, compared to BSF diet that matched that of Casein diet. Further increment of protein and reduction of cuticle in BSF-meal (BSF-2) did not further improve weight gaining rate, compared to BSF-1. Izonitrogenous Bees-based diet with lower levels of cuticle (Bees-1) did not further improve weight gaining. About 15% of the differences in weight gain between BSF and Casein were explained by differences in protein gain. Food intake was affected by diet and taking into account dietary differences in combustible energy content showed that gross energy intake of BSF-based diets matched that of Casein and was lower than that of Bees-based diets, and the later matched Chow combustible energy intake. Digestibility was also affected by diet, with BSF, Bees and especially BSF-1 diets showing lower energy digestibility compared to Casein, while BSF-2 and Bees-1 showing Casein-

matching energy digestibility. Mice from all diets seem to regulate their food intake according to dietary combustible energy content and dietary energy digestibly, resulting all together in lack of dietary differences in net absorbed energy. Hence, weight gain efficiency (weight gain per absorbed calorie) was lower for Chow and BSF, compared to Casein. These phenotypic differences in weight gain efficiency may result from dietary differences in the cost of digestion and absorption of the cuticle-rich meals or in other means of energy utilization. True nitrogen digestibility was also affected by diet, with BSF and BSF-1 diets showing Chow-matching lower true nitrogen digestibility, compared to Casein, while BSF-2 and Bees diets showing Casein-matching true nitrogen digestibility. Total nitrogen absorption was higher in Chow diet and lower in all BSF diets, compared to Casein diet that matched both Bees diets. Weight gain per total nitrogen absorption was lower in Chow and BSF diets, compared to Casein diet that matched all other diets. Protein gain per total nitrogen intake, as well as per total nitrogen absorption (i.e. net retention efficiency of dietary nitrogen), was lower in Chow and in BSF diets, compared to Casein that matched all other diets. These results are in accordance with the relatively low amino acid score of BSF diet compared to Casein. In all, these results show clear dietary benefits for using untreated Bees-meal, compared to the untreated BSF-meal, as a sole source of protein. Notably, they also show that the dietary efficiency of the Bees-meal is similar to that of a casein-based diet. Decreasing cuticle level did not affect dietary efficiency of Bees-meal but increased efficiency of BSF-meal to that of casein.

Processing of honeybee drone brood as a protein source: Enhancement of sustainability level with high-moisture- and dry extrusion technology

Magdalena Ulmer, Sergiy Smetana, Volker Heinz

Deutsches Institut für Lebensmitteltechnik e.V., Quakenbrück, Germany

Corresponding author: lena.ulmer@gmx.de

Extrusion combines several steps like mixing, cooking, kneading, shearing, shaping and forming in a single unit to a continuous process so that investment and running costs are low. The food industry widely uses it, e.g., for snacks, cereals, or products on sugar basis. Additionally, experiments to produce meat analogs (products that have similar to meat texture, mouth-feel, taste, and nutritional value) via high moisture extrusion cooking have previously proven to be successful. Also, extrusion is often indicated as a technology leading to the development of more sustainable products due to the generation of lightweight products with an extended shelf-life.

To promote entomophagy in Germany, the use of drone brood stemming from a widely accepted insect – the honeybee (*Apis mellifera*) – could be helpful. It is a particularly promising edible resource, as many beekeepers regularly remove frames with drone brood to minimize the destructive varroa mite (considered as the most damaging enemy of the honey bee) in the colonies. Usually, the drone brood then is regarded as waste.

This research combined the use of frozen drone larvae with extrusion, resulting in satisfying products (both for high moisture- and dry extrusion). The high-moisture intermediates containing up to 35% of drone brood had much lower burden for the environment (comparing to chicken meat). The most promising product is expanded texturized insect protein processed with soy concentrates via dry extrusion with 20% of drone brood that can result in a protein-rich snack (65% protein content).

Exploring the potential of honeybee (*Apis mellifera carnica*) larvae and pupae for the production of high added-value food supplements and nutraceuticals

Matjaž Deželak[1], Adriana Pereyra Gonzales[1], Lovro Sinkovič[2], Domen Jaklič[1], Slavko Bernik[1], Ažbeta Gosak[1], Drago Kočar[3], Mitja Kolar[3], Rok Kopinč[1]

[1] *Medex d.o.o., R&D Department, Ljubljana, Slovenia*
[2] *Agricultural Institute of Slovenia, Ljubljana, Slovenia*
[3] *Faculty of Chemistry and Chemical Technology, Chair of Analytical Chemistry, Ljubljana, Slovenia*

Corresponding author: matjaz.dezelak@medex.si

According to the FAO, by 2050 the world will host 9 billion people, which means that current food production will need to almost double. Edible insects have always been a part of human diets in other continents, whereas in Europe entomophagy did not became an accepted type of diet. In recent years, headlines claiming “eating insects is the future of food” and "edible insects are the next superfood” have been appearing, indicating two distinct ways of insect use: as a regular food (source of macronutrients and energy) and as a food supplement/nutraceutical (source of micronutrients and other bioactive compounds).

Using insects as food and feed has gained enormous interest in the past years, whereas the discovery of health-promoting bioactive compounds was not considered as a priority. In doing so, larvae and pupae of honeybee (*A. mellifera*) seem the preferred choice since the so-called 'Apilarnil', a drone larva homogenate, is already used in apitherapy and folklore nutrition. European Food Safety Agency (EFSA) perceives honeybee brood as an apiculture product, which means safety is

Edible bugs in European food culture: a cultural analytical approach towards overcoming the yuck factor

Claudia Manzo

Lund University, Department of Arts and Cultural Sciences, Lund, Sweden

Corresponding author: claudiamanzo93@gmail.com

Entomophagy, i.e. the practice of eating insects, has been part of human eating habits since prehistory. In many areas of the world, such as Africa, Asia and Latin America, insect-eating is ongoing and is embedded in the culinary tradition of these countries. Meanwhile in European food culture at large, bugs assumed a negative representation, being considered as pests infesting crops and perceived as dirty creatures carrying diseases. As a result, the consumption of insects is largely seen as an uncommon practice and is marked by stigma. However, in the last decades an increasing number of people in the Western world is starting to eat insects, moved by environmental concerns or curiosity.

Indeed, insect food is gaining popularity especially among those gourmets who are looking for a unique culinary experience, and turning to those luxury restaurants and companies who are not afraid of experimenting with beetles, crickets or worms and incorporating them in their recipes. At the same time, the attempts of many pioneer entopreneurs, that is entrepreneurs working to promote insects as a food delicacy, have been made difficult in Europe not only because of the cultural barriers towards entomophagy, but also because of the legal uncertainty of EU regulations about edible insects, prohibiting or restricting the production and consumption of novel foods such as edible insects.

My aim is to formulate practical recommendations on how to improve the acceptance and commercialization of insects as food suitable for human consumption, especially in European markets. In order to attain this aim, I conduct a cultural analysis of the social and cultural implications of entomophagy perception in European food culture. To begin with, I will explore the reasons behind the yuck factor, that is the disgust feeling towards the practice of eating insects, and compare these insights with experiences of regular consumers of insect food. Further, through the investigation of the online bug eaters community I attempt to profile the insect eaters and understand how they moved from not eating the so called grubs to become regular consumers of insect food. Bridging between these two contrasting perspectives, I will demonstrate how the introduction of insects in European food culture affects the notion and perception of edible and inedible foods.

Keywords: Entomophagy, edible insects, novel food, super food, edible, inedible, food culture, nutrition, sustainability, neophilia, neophobia

Willingness-to-try edible insects – The impact of consumers' perception and visual characteristics of insect dishes

Eric Barrera Albores, Isabel Schäufele, Ulrich Hamm

University of Kassel, Department of Agricultural and Food Marketing, Witzenhausen, Germany

Corresponding author: barrera.a.eric@gmail.com

The application of insects as food has substantially increased in the last years and thus their presence in the media. Most communication efforts have been focused on insects' environmental and nutritional benefits since edible insects are considered a sustainable alternative to meat. However, consumers' acceptance for edible insects still remains low. A major reason is consumers' perception of insects as disgusting due to their visual features.

How insect dishes are presented to consumers, i.e. the degree of visibility and the particular type of insect, is considered highly relevant for consumer acceptance. Even though many studies found that reducing the degree of visibility improved the acceptability, no study so far focused on the effect of the insect species within a quantitative consumer survey. The present study aimed to close this gap through the analysis of factors that improve the acceptability of edible insects measured by the willingness to try dishes that varied between two insect species (grasshoppers and mealworms) and three degrees of visibility (whole insect, smashed, processed).

The findings show that consumers' perception of insects' benefits had no relevant influence on the willingness-to-try. The most relevant aspect was the social acceptability of insect consumption. The willingness-to-try the dishes can be improved by decreasing the visibility of insects, but also by using grasshoppers instead of mealworms. Serious information and educational work is required in order to change social and cultural norms and thus the social acceptability of insect consumption.

Novel enzymes for stem cell harvest

Tanja A. Grein[1], Jasmin Leber[1], Florian Petry[1], Zdeněk Franta[2], Anne-Kathrin Pöppel[2], Katharina Merkewitz[1], Denise Salzig[1], Holger Zorn[3], Peter Czermak[1]

[1] University of Applied Sciences Mittelhessen, Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany
[2] Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources, Giessen, Germany
[3] Justus Liebig University Giessen, Institute of Food Chemistry and Food Biotechnology, Giessen, Germany

Corresponding author: tanja.a.grein@lse.thm.de

In cell therapy, the use of autologous and allogenic human mesenchymal stem cells is on the rise, incurring a demand for high quality cells for clinical applications. In contrast to the manufacturing of other therapeutics (e. g. antibodies), hMSC must remain viable and unaffected. Considering the cGMP guidelines, cell harvesting is a challenging assignment as hMSC grow strictly adherent to a surface. In stem cell manufacturing, cells are detached by enzyme treatment which can influence the basic metabolism of hMSC.

Standard enzymes, such as bovine/porcine trypsin, bear the risk of damaging cells.

The approach pursued here involves a gentler harvesting of stem cells, using novel enzymes (e.g. the insect based Jonah-like chymotrypsin from the therapeutic maggot *Lucilia sericata*, or prolyl-specific peptidase (PsP) from fungus Wolfiporia cocos).

PsP show better results in detachment time and efficiency compared to trypsin, leaving the hMSC rather unaffected. Due to a reduced influence on the hMSC metabolism, PsP is a potential candidate for cell harvesting in a dynamic cultivation system and it is undoubtedly a key to successful cell therapy.

Production strategy of an insect-derived antimicrobial peptide in the microbial expression system *Escherichia coli*

Joel Eichmann[1], Mathias Joachim[1], Doreen Gerlach[2], Peter Czermak[1]

[1] *University of Applied Sciences Mittelhessen, Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany*
[2] *Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources, Giessen, Germany*

Corresponding author: joel.eichmann@lse.thm.de

The emergence of rising antibiotic resistance in human and veterinary pathogens has been a driving force in the search for new classes of antibiotics. Antimicrobial peptides (AMPs) are multifunctional effectors of the innate immune system in nearly all living organisms and present antimicrobial activity against a range of pathogenic bacteria, viruses and fungi. A promising therapeutic AMP candidate is the insect metalloproteinase inhibitor (IMPI) inhibiting virulence-mediating microbial M4-metalloproteinases. The biomolecule IMPI was discovered in the hemolymph of larvae of the greater wax moth *Galleria mellonella* and contains five intra-molecular disulfide bonds. The supply of sufficient amounts of the AMP required for structural determination, toxicity testing and preclinical studies favor heterologous expression for the production in a cost-effective manner. Escherichia coli, as a production host of recombinant proteins, has numerous advantages over other hosts such as fast growth, rather easy handling, excellent understood genetics and metabolization of cheap media components. However, producing recombinant proteins with post-translational modifications, such as disulfide bonds, is a challenging task. Therefore, the engineered glutathione reductase (gor) and thioredoxin reductase (trxB) deficient E. coli strain Rosetta gami™ B(DE3)pLysS was applied to develop a production process with high cell densities in chemically defined minimal medium. For product purification, we developed an alternative downstream strategy based on aggregating tags and membrane filtration. Therefore, a unique combination of a thioredoxin-tag (trxA), elastin-like-polypeptides (ELPs) and a self splicing intein (ΔI-CM) was assembled using Golden Gate cloning, to facilitate a soluble AMP expression process in combination with a non-chromatographic downstream process. A tailor made chemically defined medium with optimized trace elements increased growth and recombinant production of the ELP-tagged AMP. The trxA acts as a solubility enhancer and facilitates the release into the extracellular space by simple osmotic shock procedure. Reversible temperature dependent phase transition of the ELPs enables membrane filtration steps for purification of the desired product from impurities and, after cleavage by self-splicing inteins, to separate the AMP from the tags itself. The interaction of thioredoxin-, ELP- and intein-tag facilitates scalability and economic feasibility of the IMPI production process. With the integration of process analytical technology (PAT) and a design of experiments (DoE) based upstream optimization we established a process with high potential for application in pharmaceutical industry in regard of GMP-compliance.

Impact of waste management technologies on environment: relative life cycle assessment of potato peels management with anaerobic fermentation, pigs and insects

Shashank Goyal, Sergiy Smetana, Volker Heinz

German Institute of Food Technologies - DIL e.V., Food Data Group, Quakenbrück, Germany

Corresponding author: s.goyal@dil-ev.de

The research aimed to identify a more sustainable method of food waste (potato peels) management. A research gap exists in comparative environmental impact assessment of different food waste treatment methods. It becomes questionable to determine which waste treatment method has lower environmental impacts. The environmental and economic aspects of sustainability for waste treatment via animal feeding, insect feeding, and anaerobic fermentation were studied in this work.

Life cycle assessment attributional approach was used for comparative environmental impact analysis of waste treatment methods. The data was collected from previous researches and personal communication with industry (potato processing and *Hermetia Illucens* insect farm). Functional units: 1 kg of potato peels treated; 1000-euro sale of the product; and 1 kg of protein obtained. The results showed the highest environmental impacts of potato peels waste treatment from pigs (175.74 µPt) as compared to insects (16.9 µPt) and anaerobic digestion (20.3 µPt). Alternative calculations on functional units of gross sale amount and protein product (only for pigs and insects) demonstrated that insects were more sustainable than pork and biogas.

“Fair” comparison of potential bio-treatment scenarios with *H. illucens*, pigs and anaerobic fermentation indicated that both insects and fermentation are more beneficial and efficient for waste treatment than pork, while insects also can generate higher protein amount than pigs from the same amount of potato peels. It is expected that the impact of potato peels treatment will be “diluted” with other products as feeding animals usually require more complex diets.

Poster Presentations

Soluble production of the insect-derived recombinant antimicrobial peptide lucimycin in the novel expression host *Vibrio natriegens* Vmax express

Joel Eichmann[1,2], Doreen Gerlach[3], Tobias Weidner[1], Peter Czermak[1,2,3,4]

[1] *University of Applied Sciences Mittelhessen, Institute of Bioprocess Engineering and Pharmaceutical Technology, Giessen, Germany*
[2] *Faculty of Biology and Chemistry, Justus Liebig University, Giessen/Germany*
[3] *Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Project group Bioresources, Giessen, Germany*
[4] *Department of Chemical Engineering, Kansas State University, Manhattan/USA*

Corresponding author: joel.eichmann@lse.thm.de

The worldwide increasing demand for antibiotics and increasing number of resistances triggered the identification of many antimicrobial peptides (AMPs). However, due to their short sequences and toxic effects recombinant production of AMPs in E. coli is difficult and often results in no or insoluble product.

The gram-negative bacterium *Vibrio natriegens* is the fastest growing known organism with a doubling time of less than 10 min. The novel engineered strain *V. natriegens* Vmax Express, carrying the T7 expression system is a suitable and promising expression system for recombinant proteins, analog to E. coli.

Here we report the soluble recombinant production of the antimicrobial peptide BR021 from *Harmonia axyridis*, which has shown to be active against gram-negative bacteria. We applied a streamlined high-throughput screening to identify high-producers. Using multiplex Golden Gate cloning, we generated a plasmid library with 200 combinations of synthetic ribosome binding sites, secretion signals and fusion proteins for affinity chromatography. In a second cloning step, the library was assembled with a promoter and BR021, tagged with a marker for fluorescent analysis. The resulting library was expressed in *E. coli* BL21 and *V. natriegens* Vmax. Cells showing a strong fluorescent signal were sorted by FACS and cultivated again. Multiple cycles of growth, production and sorting were performed. Subsequent analysis of plated cells revealed single clones of a high-producer, expressing soluble BR021 fused to thioredoxin and a His-Tag in *V. natriegens* Vmax.

Altogether, these results show that *V. natriegens* Vmax is a suitable system for the soluble expression of insect-derived antimicrobial peptides.

Detection of aflatoxin B1 in different life stages of Black Soldier Fly (*Hermetia illucens*)

Annalisa Grisendi, Valeria Listorti, Francesco Defilippo, Giorgio Fedrizzi, Michele Dottori, Paolo Bonilauri

Lombardy and Emilia Romagna experimental Zooprophylactic istitute, Reggio Emilia, Italy

Corresponding author: francesco.defilippo@izsler.it

Mycotoxin-contaminated cereals (e.g., maize, wheat, barley) and nuts (e.g., peanut, walnuts) can be deleterious to humans and can reduce animal health and production. The mycotoxin presence in these products is monitored and, when concentrations are above maximum allowable limits, products are downgraded or removed from the food chain. Insects can be used as food by both humans and animals so they contribute to food security. There is growing interest in larvae of the black soldier fly (*Hermetia illucens*, BSF). BSF larvae can be sustainable proteinaceous feed ingredients for pigs, poultry, and fish, as well as alternatives to conventional sources (soybean meal and fishmeal) with associated environmental and societal issues. The mycotoxin tolerance of these insect species is unknown. In nature, the larvae of BSF usually thrive in various decomposing materials and are commonly reared on diets of approximately 70% moisture, and kept at 28°C. Both these conditions promote bacterial and fungal growth, therefore the current study investigated the potential accumulation of Aflatoxin B1 (AfB1) in larvae (L3 and L4), pre-pupae and pupae of the BSF.

Insects were reared under controlled conditions (RH 70%, photoperiod 14:10 h (L:D) and temperature of 25°C) on two substrates: Gainesville diet for the control group and a mycotoxin contaminated diet, enriched with peanuts containing naturally high concentration of AfB1 (38 µg/kg), for the contaminated groups. AfB1 concentration in contaminated diet was of 3 µg/kg.

At the end of the exposure period the insects were separated from the residual material, washed, killed by freezing and analyzed (LC-MS/MS).

The results showed that the concentrations of AfB1 on the larvae reared on the contaminated diet remain far below 1 µg/kg (L3 = 0.2 µg/kg, L4 = 0.1µg/kg) and in pre-pupal and pupal stage the concentration of mycotoxin further decrease under the detection limit of the method (< 0.05 µg/kg). Finally, in the residual diet high levels of AfB1 were detected (from 5.1 to 6.5 µg/kg).The applied rearing conditions were successful in supporting the development of BSF with high survival rates. The BSF larvae do not accumulate AfB1, this confirms the results obtained in previous studies and leads us to suppose that mycotoxins are present only in the gut and not in other insects tissues. The slightly higher concentration of AfB1 in the diet at the end of the experiment could have been due to the fact that larvae not accumulating AfB1 and reducing the substrate during their growing, have produced a concentration of the AfB1 in the residual diet.

Bioaccumulation factor of cadmium in the different life stages the Black Soldier Fly, *Hermetia illucens*

Francesco Defilippo, Annalisa Grisendi, Valeria Listorti, Giorgio Fedrizzi, Michele Dottori, Paolo Bonilauri

Lombardy and Emilia Romagna experimental Zooprophylactic istitute, Reggio Emilia, Italy

Corresponding author: francesco.defilippo@izsler.it

There is an urgent need to increase the supply of sustainable protein sources to be used in animal feed and the use of insect's protein provides a potential alternative to protein crops and fishmeal. For instance, fly larvae contains high levels of digestible protein with key amino acids, comparable with those found in high value protein sources such as soybeans, and could be recommended for use in animal feed. Among the insect species, there is an increasing interest in rearing black soldier fly larvae (BSF, *Hermetia illucens*). Larvae of this species can be used for biodegradation of organic waste and are a promising feed source for animal nutrition, being rich in protein. However, studies investigating safety aspects of the use of black soldier fly as feed are scarce. The aim of this study was to investigate the potential bioaccumulation of cadmium from the feeding media to the larvae, pupae and adults of BSF. Two sets of experiments were conducted in order to investigate:

1) Bioaccumulation Factor (BAF - metal concentration in the body divided by metal concentration in the feeding media) in BSF reared on substrate contaminated in all larval stages;

2) BAF in BSF reared on contaminated substrate until the 3rd larval instar and transferred subsequently on control diet.

BSFL were reared under controlled conditions (RH 70%, photoperiod 14:10 h (L:D) and temperature of 25°C) on two substrates: Gainesville diet for the control group and a cadmium contaminated diet, enriched with horse liver containing naturally high concentration of cadmium (1.303 mg/Kg), for the contaminated groups. In all experiments, larvae, prepupae, pupae and adults accumulated cadmium. The BAF of cadmium in larvae was between 3,98 and 2.33, in prepupae 3.54 and in pupae 2.39. Heavy metal BAF in adults was lower than in other life stages (1.25). In the 2nd experiment where BSFL were reared on contaminated substrate until the 3rd larval instar and then transferred on control diet, the BAF was much higher than the levels found in experiment 1 (4th larvae instar 11.27, prepupae 8.08 and pupae 6.19). So that, the Cd accumulated by larvae until the reaching of the 3rd larval instar is slowly eliminated by the larvae even though transferred on clean control diet. Moreover the variations of protein source in control diet (liver vs alfa alfa) has stretched the development time of the BSF in the experiment 2 and therefore the feeding time for each life stage in the experiment 2 was much longer than in the experiment 1, causing a further accumulation of the cadmium naturally present in control diet. Development time from hatching of the larva to the prepupal, pupal and adult stage generally increased with the increasing of heavy metal concentration in

the feeding media. Our study reveal an high capability of BSF larvae and prepupae to accumulate cadmium and this could potentially limit their use for animal feed production. A broader understanding of the occurrence of this undesirable substance in processed larvae products is needed to assess feed and food safety.

Exploring the use of the larval immunity to manage the microbiota of Black Soldier Fly larvae and increase their microbial safety during rearing – a literature evaluation

Jeroen De Smet, Enya Wynants, Leen Van Campenhout

KU Leuven, De Dijken, Dessel, Belgium

Corresponding author: jeroen.desmet@kuleuven.be

The Black Soldier Fly (BSF, *Hermetia illucens*) has become one of the most important insects in the world for bioconversion, as its larvae are able to valorise low quality organic waste streams (e.g. household organic waste). Such waste streams often have a high microbial load, raising questions on the microbial safety of the larvae reared on these substrates. We performed an extensive literature search1 to explore how these larvae could cope with the presence of various micro-organisms and the impact their presence will have on the growth and microbial content of the larvae. Our search points at the existence of a set of currently hardly explored mechanisms in place that could avoid the microbial community in the BSF larvae from being taken-over by specific bacteria on a range of substrates, potentially in different stages of decay. This is evidenced by the observation that the counts of two pathogens, Escherichia coli O157:H7 and Salmonella spp., were found to be reduced in the substrate in the presence of the larvae2. However, the underlying mechanisms are not yet identified. At the same time, larval immunity is found to react to the presence of micro-organisms with the production of antimicrobial peptides that could kill these micro-organisms3. An overview of these findings will be presented, combined with the needs for future research to explore to what extent food pathogens, potentially present in the substrate, can colonize the BSF microbiome, and if and how modulation of BSF immunity will impact colonization.

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Detecting fine plastic contamination in the feed and body of the larvae of *Hermetia illucens*

Ward Tollenaar, Eric Schmitt, Aman Paul

Protix Biosystems, Research and development, Dongen, Netherlands

Corresponding author: Ward.Tollenaar@protix.eu

Secondary organic streams that are attractive feed sources for the larvae of *Hermetia illucens* may contain fine plastic particles that are difficult to detect by the naked eye. These fine plastic particles pose a definitive risk of being consumed by the larvae and entering the food chain. This makes screening of such feed streams for plastic contamination extremely important. Powerful methods exist for detecting plastic residues in materials, but many are expensive or too complicated to be routinely used in a production environment. This poster presents work in progress on accessible methods that might be used in a production environment. It highlights where difficulties have arisen and points to directions where further research could be directed to develop powerful and efficient methods.

Separation of faeces, exuvia and feed residues from *Tenebrio molitor* larvae

Andreas Baur, Mohamed Hussein, Antonio Delgado

FAU Erlangen – Nuremberg, Institute of Fluid Mechanics, Erlangen, Germany

Corresponding author: andreas.baur@fau.de

The separation of larvae from unwanted components is a necessary process step to comply with hygiene standards. It is achieved by an air classifier, an alternative provess step beside sieving. The separation system is based upon a set of 11 zig-zag inclined paths to increase the separation efficiency, sorting the different contents of a rearing box. For this, the settling velocity of the different contents of an rearing box has to be calculated. The settling velocity of fully grown larvae is 13 m/s, the settling velocity of exuvia, faeces and feed residues is between 1-5 m/s. The tuning of the ascending air between 7-10 m/s will lead to separation of the larvae from the remaining contents. A further aim is the separation of eggs or young larvaes from the growth substrate. The challenging part is the almost similar settling velocity of *Tenebrio molitor* eggs and the growth substrate (wheat bran). By changing the growth substrate, air velocity and the number of inclined path the separation should be made possible.

Predicting the number of offspring in *Tenebrio molitor*

David Deruytter, Carl Coudron, Jonas Claeys, Stefan Teerlinck
Inagro, Rumbeke-Beitem, Belgium
Corresponding author: david.deruytter@inagro.be

In an industrialized mealworm farm it is important to know the number of individuals in each container as early as possible in a fast and reliable way. If this information is combined with a growth chart, it can lead to a more efficient feeding regime. This in turn can result in a faster growth and more revenue. Therefore in this research different possibilities were assessed to predict the number of offspring (*Tenebrio molitor*) based on the beetle density and or egg density.

Three different techniques were assessed to predict the final harvest. In the first two techniques the beetles were allowed to deposit their eggs on the bottom of a black 600*400 mm container. A full factorial design was assessed with eleven beetle densities (between 2.2 and 250 mg beetles/cm²) and five deposit times (1, 2, 4, 7 and 14 days). In the first method a linear regression model was constructed based on the density, deposit time and interaction to predict the number of offspring. In the second method image processing was used to calculate the area covered by eggs of the container. This was then used as a proxy of number of offspring. Finally in the last method attempts were made to separate the eggs from the substrate. Hence, in theory perfect dosing of the containers with a known number of eggs would be possible.

The initial results indicate a clear correlation between the beetle density, deposit time and the final number of mealworms. Furthermore, it was possible to construct a formula based on a single new parameter: beetle-days, similar to the concept of degree-days. Hence, doubling the number of beetles or the deposit time has the same effect on the final harvest. In the final model it was possible to predict a priori the number of mealworms up to an accuracy of a factor 1.5. In the second method, using ImageJ, it was possible to determine the area covered by the eggs and construct a model to correlate this number to the final number of mealworms. This may certainly be useful in automated insect production systems to a posteriori determine the number of mealworms or to check the first method (e.g. changes in egg deposit due to an illness). However, it is very important to handle the crates in a similar way so that any debris sticking to the bottom is removed in a similar way. Finally, it was possible to gather individual eggs when beetles deposited through a mesh in flour. However, this results in an additional handling step of the delicate eggs which may result in additional mortality or costs (handling time). Furthermore we were not able yet to separate the eggs perfectly as some debris was still present and flour sticking to the eggs. In conclusion, it is possible to determine the final harvest at a very early stage via the 3 presented techniques potentially resulting in more optimal feeding and growth.

Wheat diets supplemented with calcium iodate lead to altered growth performance and iodine accumulation in *Tenebrio molitor* larvae

Anna-Kristina Marel, Lucas Hartmann, Marcel Lieber, Ann-Katrin Meinhardt, Alexandra Müller, Ralf Greiner

Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Department of Food Technology and Bioprocess Engineering, Karlsruhe, Germany

Corresponding author: anna-kristina.marel@mri.bund.de

Within the next decades, insects such as the *Tenebrio molitor* larvae (mealworms) could play a major role as alternative food source for the growing population. Their ability to transform low value processing by-products into protein- and fat-rich biomass is a great chance for sustainable food production systems. Key parameters for ideal growth performance need to be identified and optimized in industrial insect farming. Since there is strong evidence that insects tend to accumulate contaminants from their feed, one important factor of enhancing rearing conditions is the substrate presented to them.

In this study, we investigated the effect of different wheat based diets on weight gain, death rate and weight of pupae of mealworms over eight weeks. Additionally, mealworms were fed calcium iodate supplemented diets to explore their capability of enriching the essential trace element. Mealworms grown on wheat bran and wheat semolina bran showed comparable growth performance regarding larvae and pupae weight as well as comparable death rate. The iodine supplemented diet led to lower medium individual larvae weight, however, the percentage of dead mealworms was clearly lower within the first four weeks. Already after four weeks, the mealworm iodine content has increased fourfold and reached almost the level reported for pork. Thus it was shown, that iodine contents in mealworms comparable to conventional meat sources can be obtained.

The better understanding of breeding conditions and usage of low quality diets from processing by-products as feed for insects can contribute to ensure the world food supply in a sustainable framework.

Storable feed substrates in the feeding of field crickets (*Gryllus bymaculatus*) and desert locusts (*Schistocerca gregaria*)

Brigitte Paulicks, Philipp Straub, Wilhelm Windisch

Technische Universität München, Lehrstuhl für Tierernährung, Freising, Germany

Corresponding author: paulicks@wzw.tum.de

The Mediterranean Field Cricket *Gryllus bymaculatus* and the Desert Locust *Schistocerca gregaria* are edible insects contributing to food protein in many human communities. However, they arise from natural catch or from rearing on base of solely fresh feed materials, which makes the systems vulnerable in terms of food security and food safety. Storable and hence controllable substrates could overcome this disadvantage. This pilot study focuses on the possibility to replace fresh by storable feed substrates for rearing these insects.

Methodology:

Crickets and locusts were held in cages containing each 250-350 cricket nymphs (n=12 cages) or 220-330 locust nymphs (n=21 cages) upon hatching. Then, the animals received cage-wise different diets consisting of five storable substrates: corn, soybean meal, cowpea leave, corn stover, and vitamin enriched dried carrots. Crickets received a combination of corn and cowpea leave („Starch"-diet, n=6) or soybean meal and corn stover („Protein/Fiber"-diet, n=6). Locusts were fed the „Starch"-diet (n=6) or „Protein/Fiber"-diet (n=6) alone or in combination with vitamin enriched carrots („Starch/Carrot"-diet, n=3; „Protein/Fiber/Carrot"-diet, n=3), and a combination of cowpea leaves, soybean meal and vitamin enriched carrots („Protein/Carrot" diet, n=3). Total feed consumption, harvested insects and total excrements were monitored cage-wise. Animals and excrements were frozen and analyzed for nutrient contents. Apparent digestibility of feed dry matter (DM) was estimated based on excrement DM. Statistics included ANOVA within insect species using diet as treatment and individual cages as replicates.

Results:

Crickets gained biomass well when fed on „Starch"-diet but not on „Protein/Fiber"-diet. Consequently they transformed feed DM into body DM more efficient when fed on „Starch"-diet than fed on „Protein/Fiber"-diet (15% vs. 10%, $P<0.01$). Digestibility of feed DM was higher with „Starch"-diet than with „Protein/Fiber"-diet (55 vs. 42%, $P<0.01$) whereas conversion of digested feed DM into body DM showed the same efficiency. Feed nitrogen was transformed more efficient in crickets fed on „Starch"-diet compared to „Protein/Fiber"-diet (43 vs. 27%, $P<0.001$). Locusts fed on „Starch"-diet and „Protein/Fiber"-diet failed to perform. Adding carrots did not improve growth on „Starch/Carrot"-diet, but locusts gained biomass receiving „Protein/Fiber/Carrot"-diet. Feed DM transformation was more efficient with „Protein/Fiber/Carrot"-diet (16%) and

„Protein/Carrot“-diet (20%) than with „Starch“-diet (11%, P<0.01) whereas DM digestibility did not differ. Transformation of digested feed DM into body DM was less efficient with „Starch“-diet (16%) than with „Protein/Carrot“-diet (39%, P<0.05) and „Protein/Fiber/Carrot“-diet (28%). Obviously, locusts fed on „Starch“-diet could digest the feed, but they were not able to utilize the nutrients efficiently for growth (probably due to protein deficiency). Corresponding nitrogen transformation of „Protein/Fiber/Carrot“-diet was superior to „Starch“-diet (49% vs. 36%, P<0.05).

Conclusion:

Crickets as well as locusts accepted the offered substrates as feed. Therefore, these substrates may replace fresh feed materials and may thus improve efficiency and safety of insect production systems. Indeed, certain feedstuff combinations revealed nutritional limitations. They might serve as model diets to derive nutrient (e.g. protein, amino acids, vitamins) requirements for crickets and locusts used as edible insects.

Optimization of Black Soldier Fly artificial reproduction

Bertrand Hoc, Rudy Caparros Megido, Frédéric Francis

Gembloux Agro-Bio Tech-University of Liège,
Entomologie fonctionnelle et évolutive, Gembloux, Belgium

Corresponding author: bertrand.hoc@doct.ulg.ac.be

The black soldier fly (BSF), *Hermetia illucens* (L.) (*Diptera: Stratiomyidae*), is an endemic fly species from the tropical, subtropical and warm temperate zones of America. This saprophagous species relies on its environment where it finds the decomposing matter that the larvae use to grow. The polyphagous diet and the macronutrient quality (mainly lipids and proteins) of these larvae make them excellent candidates for various applications such as waste and organic material management, incorporation in animal and pet feed or alternative energy source. Although rearing development in temperate regions requires artificial processes to continuously produce high quality eggs and larvae, few studies have been conducted on the mating and oviposition processes governing the BSF reproduction. Research conducted in semi-artificial rearing conditions in Korea showed that the number of mating varied according to the season. It has been speculated that this behavior could be due to differences in the intensity of sunlight caused by the change of seasons. This study aims at evaluating the influence of sex-ratio, density and nychthemeral cycle on BSF reproduction. In order to tackle this problematic, an artificial set up for oviposition to collect eggs has been developed and two populations with opposite sex-ratios (male predominance and female predominance) were selected. Their respecting oviposition rates have been tested for five different densities. The oviposition rate was also evaluated over four nychthemeral cycles (2, 6, 12 and 18h of daily light) with the sex-ratio and at the density that resulted in the highest oviposition rate in the previous experiment (6500 individuals/m^3). The results revealed a significant influence of the nychthemeral cycle on the oviposition rate of BSF. This experiment allows to improve our understanding of the artificial reproduction of BSF, giving us the ability to intensify experiment al rearing models offering high quality and high quantities of eggs.

Towards insect bioeconomy in Finland

Susanne Heiska, Vilja Varho, Johanna Kohl
Natural Resource Institute Finland, Luke, Joensuu, Finland
Corresponding author: susanne.heiska@luke.fi

Insect industry is growing rapidly in Finland. Finland accepted insects as food in September 2017, and as feed already in July 2017, with some restrictions. The first food products containing insects were in the market in November. A number of new players have recently joined the industry in Finland, and the number of registered insect farmers is approximately 40. Insect production is seen as a promising new production line for farmers, with potential to keep the countryside viable. In addition, new insect rearing technologies are expected to contribute to new solutions in agro-industrial symbioses, and to food production in the cities. In the future, insect industry could provide new business opportunities for producers with different competition strategies, searching for advantage either from specialization or mass production. However, the sustainable and balanced growth of insect industry requires a common understanding about the current state and the desired future of insect industry. A big picture of the factors preventing and driving the sectoral changes is also needed. Thus, a road map will be prepared, serving as an anticipation tool for various actors in the industry. An iterative, participatory and transparent process will be built up in order to collect existing know-how, to create future visions considering regional strengths and different factors affecting the future, and to refine regional views into a nationwide road map. Finally, the big picture will be cascaded into regions by utilizing regional stakeholders.

Optimization of hatching and neonate survival of *Hermetia illucens* larvae

Lotte Frooninckx[1], Meggie Van Peer[1], Liesbeth Vogels[1], Ann Wuyts[1], Enya Wynants[2], Jeroen De Smet[2], Leen Van Campenhout[2], Sabine Van Miert[1]

[1] *Thomas More University College of Applied Sciences, RADIUS, Geel, Belgium*
[2] *KU Leuven, Lab4Food, Geel, Belgium*

Corresponding author: lotte.frooninckx@gmail.com

When rearing black soldier fly larvae, highest mortality is usually observed during the first days after harvesting the eggs. Poor hatch and neonate survival is most likely due to microbial development which is caused by the high humidity necessary to keep the substrate moist 1–3. In this study, a harvesting protocol and feeding substrate was developed to nurse this fragile phase of black soldier fly larvae. First, several egg traps and egg laying substrates were investigated in order to obtain a standard harvesting protocol. Preservability of neonate feeding substrates consisting of varying concentrations of chicken meal, agar and methyl paraben were tested by analyzing changes in water activity, dry matter, pH and microbial load after incubation at 4°C and 28°C. Consequently, eggs were incubated at 28°C and 80% RH on these feeding substrates to investigate survival of the larvae and their influence on the water activity, dry matter, pH and microbial load of the substrates.

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Technical basis for the small-scale production of Black Soldier Fly, *Hermetia illucens* meal as fish feed in Benin

Armel Gougbédji[1], Rudy Caparros Megido[1], Philippe A. Lalèyè[2], Frédéric Francis[1]

[1] *Gembloux Agro-bio Tech/University of Liege, Agro Biochem, Passage des Déportés, Gembloux, Belgium*
[2] *Faculty of Agricultutral Sciences/University of Abomey-Calavi, Management of natural ressources, Abomey-Calavi, Benin*

Corresponding author: gougbemel@yahoo.fr

Producing fish of good quality and at a lower cost is the major objective of fish farmers. In Benin, the feeding of farmed fish is a major predicament. The food composition suffers from the lack of a reliable sources of protein. *Hermetia illucens* (*Diptera: Stratiomyidae*) larvae could offer a real solution as they have already been used in several countries in animal production. However, the species is little known in Benin and breeding techniques are ignored by producers. The present study aims at establishing a technical reference for the breeding of this fly in Benin. A larvarium (42 cm x 29 cm x 25 cm) equipped with an automated prepupa harvesting system was manufactured for the rearing of the larvae. A total of 36,000 larvae were used in the trials. Three densities (1 larva/g, 2 larvae/g, 3 larvae/g) have been tested in triplicate in 2 kg of chicken feed in order to determine the optimal load density. The experiment lasted 12 days during which growth tests (e.g. weight measures) were conducted. The quantities of fly meal produced from each treatment were evaluated as the total cost of production. Results show that the larvarium designed is suitable for *H. illucens* larval rearing and that the automatic prepupa harvesting system is efficient. The load density determined as ideal for good larval growth is 2 larvae per gram of chicken feed. With 40 g of 1-week-old larvae, it is possible to produce about 500 g of insect meal. The overall production cost came to roughly $ 265, which makes it quite affordable. The proposed rearing system is a high-yield one and is within the reach of any fish farmer or farmer in general. Other types of substrates such as restaurant waste, manure or agricultural co-products could be explored in order to replace the chicken feed.

Key Words: *Hermetia illucens*; technical reference; optimal larval density; cost

Isolation and characterization of lipids, proteins and chitin from Black Soldier Fly (*Hermetia illucens*) larvae, prepupae and pupae

Bert Verbinnen[1], Ilse Van de Voorde[2], Guido Aerts[2], Johan Claes[3], Mik Van Der Borght[3]

[1] *Thomas More, RADIUS, Geel, Belgium*
[2] *University of Leuven, Department of Microbial and Molecular Systems, Ghent, Belgium*
[3] *University of Leuven, Department of Microbial and Molecular Systems, Geel, Belgium*

Corresponding author: mik.vanderborght@kuleuven.be

Black soldier fly larvae (*Hermetia illucens*) are suitable for mass production and have the potential to efficiently convert a wide variety of organic waste streams into valuable proteins, fats and chitin. Because of their good nutritional profile they can be used in the production of compound feeds. Apart from using whole insects, extraction of its valuable components may result in a more refined product that can be applied as a high-value ingredient in compound feeds, but also in technical applications as surfactants, plastics, fibers, etc. In order to use black soldier flies as a source of valuable biomass we developed a fractionation procedure for the sequential isolation of lipids, proteins and chitin from the different life stages (larvae, prepupae and pupae) of *Hermetia illucens*. Moreover, a chemical characterization of the obtained biomolecules was performed. Our research revealed that the protein solubility profile of the larvae, pre-pupae and pupae are very similar, exhibiting maximal and minimal solubility at pH 11.0 and 4.0 respectively. These resembling solubility profiles allowed us to use the same separation procedure for the different life stages, although their proximate composition clearly differed. Both the fatty acid and amino acid profile of the isolated fats and proteins appeared to depend on the life stage. The isolated chitin did not contain any impurities and showed an acetylation degree of about 90%. The results of our study provide a scientific basis for the comprehensive utilization of *H. illucens* components in several industrial processes and applications.

Edible insects used by the indigenous people in the Ucayali, Amazonian Peru

Ruben Casas Reategui[1], Pablo Pedro Villegas Panduro[2], Zbyněk Polesny[1]

[1] Czech University of Life Sciences Prague,
Faculty of Tropical AgriSciences, Praha, Suchdol, Czech Republic
[2] Universidad Nacional Intercultural de la Amazonía,
Facultad de Ingeniería y Ciencias Ambientales, Yarina, Peru

Corresponding author: rcasasr@yahoo.com

Different insects such as beetles and ants, contribute to the protein and micronutrient intake of numerous human populations in many parts of the world including Peru. In the Peruvian Amazon, the insect represent a relevant food resource used as an alternative or complementary source of protein by indigenous communities. The study was conducted in eight communities of two ethnic groups (4 Shipibo, 4 Ashaninka) in the Ucayali Region. The main objective was to document the traditional knowledge on the use of insects as a food, and to characterize collection and consumption patterns among the communities. Samples of insects investigated were collected and preserved in vials with 70% alcohol and subsequently they were determined taxonomically. The data were collected based on thorough, semi-structured interviews with 63 (Shipibo) and 64 (Ashaninka) informants. The Shipibo people eat 4 insect species, while for the Ashaninka 6 species of insects are considered as food. The most culturally important specie is *Rhynchophorus palmarum* L. with a salience index (0.95) in Shipibo and (0.65) in Ashaninka. The edible insects are collected and consumed according to the seasonal availability. Depending on the species, only certain developmental stages are consumed. The preparation of insects for the consumption involves mainly fried, roasting and boiling. The Shipibo listed the exclusive consumption of Coleoptera while the Ashaninka, in addition to those Coleoptera, also reported the consumption of Lepidoptera. Projects on the sustainable production of insects should be promoted in the region, to spread the nutritional value of this traditional food.

Key words: ethnobiology, food, traditional knowledge, entomophagy, Amazon

How oven-drying and freeze-drying influence the nutritional composition of blanched *Ruspolia differens*

Tengweh Forkwa Fombong[1], Mik Van Der Borght[2], Jozef Vanden Broeck[1]

[1] *KU Leuven, Biology-Division of Animal Physiology and Neurobiology, Leuven, Belgium*
[2] *KU Leuven, Department of Microbial & Molecular Systems, Geel, Belgium*

Corresponding author: tengweh.fombong@kuleuven.be

Ruspolia differens plays an important role as a food source across Sub-Saharan Africa, being consumed as a delicacy in both rural and urban areas. Sun or commercial drying usually preserves it after blanching. Two commonly-employed commercial drying methods are oven-drying and freeze-drying. To convince consumers to accept insect products from different processing (drying) methods, an investigation of their effects on nutritional constituents is essential. Therefore, the influence of the above two drying methods employed after blanching, on the proximate, fatty acid, amino acid, vitamin B12 and mineral composition of the samples was determined.

No significant differences were observed between both drying methods in all nutritional parameters examined, i.e., proximate ($P > 0.98$), mineral ($P > 0.78$), amino acid and fatty acid ($P>0.79$). Results also indicated high lipid contents (36%), and significant protein levels (33% - 46%). Oleic acid (44%) was the dominant fatty acid, while the presence of arachidonic (0.6%) and docosahexaenoic acid (0.21%) suggests that this grasshopper species is a source of polyunsaturated fatty acids. All essential amino acids were present with glutamic acid (124 mg/g protein) the highest. Calcium (896–1035 mg/100 g) and potassium (779–816 mg/100 g) were high among the minerals while the presence of trace elements iron (217–220 mg/100 g) and zinc (14.2–14.6 mg/100 g) advocates their use in combatting such deficiencies. Vitamin B12 content was 1.06 µg/100g. Oven-drying *Ruspolia differens* delivered the same nutritional quality as freeze-drying post blanching. Thus, both approaches can be adequately used to formulate insect-based food products.

Insects' proximate composition and nutritional characteristics as feed, according to biological phase: some descriptive statistics from a review

David Meo Zilio, Monica Guarino Amato

Council for Agricultural Research and Economics (CREA), Livestock Production and Aquaculture (CREA-ZA), Monterotondo Scalo (Rome), Italy

Corresponding author: david.meozilio@crea.gov.it

Insects for feed are object of growing debate. Their suitability has still to be fully accepted. Insects' composition is variable depending on order, species and biological phase and could be modified via manipulation of rearing substrates and growing conditions. This work aimed to describe the variability and to highlight possible differences in composition and nutritional quality of some insects, reared under varied condition and considering the biological development stage (from the larval status to the adult). Data on chemical composition, fatty acids, mineral, amino acids (AA) and vitamins content were collected from 60 original papers out of 150. Four species, recently included in the EU regulation on animal protein (893/2017), *Acheta domestica* (AD), *Tenebrio molitor* (TM), *Hermetia illucens* (HI), *Musca domestica* (MD) were considered. Data not consistent or not sufficiently described were excluded. Following descriptive statistics were calculated: mean, median, coefficient of variation (CV), range, standard error (SE). Crude protein content ranged between 68.33% (AD, adults) on dry matter (DM) basis and 42.99% (HI, pupae) with a coefficient of variation included between 2% and 31%. TM pupae ranked first for fat (34.72%) and in general, adults of the different species contained less fat (e.g. TM 16.99%). CV was quite low and always < 50%. Minerals and ash of insects are generally related to the diet. Anyway, the highest ash content was found for AD nymphs (19.72%), but only three observations were available and CV was high (112%). TM larvae showed an even higher CV for ash (135%, n=21). MD larvae content (11.35% DM, n=29, CV=32%) resulted double than that of pupae (6.87%, n=6, CV=18%). The same trend seemed to be confirmed for TM, even though the values were lower in magnitude (5.29 vs 3.3%). However, in this case only two records contributed to the mean calculation of pupae ash content. Whilst, for HI, the content was almost the same between larvae and pupae (about 11% DM). The lowest value was found in AD adult (5.67%, n=7, CV=0.35). Since ash content may reduce the nutritional value of the feed, those aspects could have a practical impact in diet formulation for poultry, pets and livestock in general. As for minerals, the higher dispersion (CV>100%) pertains Ca, Fe, Cu, Zn, Mn. HI contains much more calcium than the other species both at larval and pupae phase. Hi larvae showed an interesting Ca/P ratio (3:1) while for all other categories of insect, excluding HI pupae, this ratio shifted towards P. Crude fiber and nitrogen free extractive were not included in this discussion owing to the still debated and not yet univocally accepted method for fiber and chitin determination in insects and for their interpretation. HI, larvae and pupae, ranked also first for saturated fatty acids content (about 60%), with a very low CV, but omega-6/omega-3 of HI larvae

is in absolute the most favorable (7.51). The most unbalanced ratio towards the omega-6 group was found for TM larvae (53.36) even though the CV is quite high (95%, n=22) and fatty acids are supposed to be very diet dependent. Generally, CV was always low for saturated/unsaturated ratio and it seems independent from the development stage. Among the most limiting AA, all considered insect categories showed good content of lysine (comparable to soybean meal), a content of methionine greater or equal than soybean meal, low content of tryptophan apart from TM adult (1.27% of total amino acids, but unfortunately we got only one observation). The most variable AA were: tryptophan (CV up to 141% in MD larvae) and cysteine with a CV ranging between 72% for MD larvae and 141% for AD adult. Orthopteran nymphs showed a low index of variability for AA but only two observations were given. The highest CV for methionine was reported for HI larvae (74%). Only few data about vitamin content were available on the current literature. Consequently, we reported just some records related to TM and AD in our research. Given this assumption, the presented data need to be further investigated. Anyway, it seems worthwhile to mention the interesting potential antioxidant profile of AD. AD adult, on the base of one observation, would contain a not negligible amount of retinol (0.28 mg/kg, n=1), slightly lower than milk, and of tocopherol (44.58 mg/kg, n=3). AD nymphs, on the other hand, showed the highest content of tocopherol (63.71 mg/kg, n=3) and ascorbic acid (206.57 mg/kg, n=2). Those last values are comparable to a vegetable like tomato. In conclusion, the high data dispersion within species and categories supports the possibility to get specific and balanced feed through the right choice of insects, substrates, treatments and growth phase. In this perspective, the inclusion of different insect derived products in the formulation of feed has also to be considered. Further in deep studies are needed, to confirm these hypotheses and to set up protocols in order to optimize the nutritional value as a function of target species.

Comparison of suitable drying techniques on nutritional quality of mealworms (*Tenebrio molitor*)

Nina Kröncke[1], Claudia Keil[2], Sandra Grebenteuch[3], Verena Böschen[4], Sebastian Demtröder[1], Andreas Thünemann[5], Lothar Kroh[4], Hajo Haase[3], Rainer Benning[1]

[1] University of Applied Sciences Bremerhaven, Food technology and bioprocess engineering, Bremerhaven, Germany
[2] TU Berlin, Institute of Food Technology and Food Chemistry, Food Chemistry and Toxicology, Berlin, Germany
[3] TU Berlin, Institute of Food Technology and Food Chemistry, Department, Food Chemistry and Analytics, Berlin, Germany
[4] Research Institute of Feed Technology, of the International Research Association of Feed Technology e.V. Braunschweig-Thune, Germany
[5] Federal Institute for Materials Research and Testing (BAM), Berlin, Germany

Corresponding author: nkroencke@hs-bremerhaven.de

The yellow mealworm (*Tenebrio molitor* L., *Coleoptera: Tenebrionidae*) is an edible insect, due to its ubiquitous occurrence it is well suited for an industrial-scale production (EFSA 2015, VAN HUIS 2013). Drying is an important preservation step in industrial insect production and processing. Within this study the effects of different drying technologies (freeze drying, microwave drying, rack oven drying, vacuum oven drying, fluidized bed drying) and process parameters on the fatty acid composition, lipid peroxidation and the appearance of volatile compounds in *Tenebrio molitor* larvae were investigated. Moreover, larvae were analyzed for total zinc values as well as the bioaccessibility of this essential mineral upon in vitro digestion.

Currently, the information about the stability of functional and nutritional components in mealworms during drying is still limited. Deepening the knowledge of process-induced changes of mealworm`s quality is urgently needed to improve drying techniques, and therefore to reduce undesirable properties of *Tenebrio molitor* while maintaining the required nutritional characteristics.

Effects of a complete substitution of soybean meal by insect meal (*Hermetia illucens*) in meat-type chicken diets

Susanne Velten, Carmen Neumann, Frank Liebert

Georg-August-University Goettingen, Department of Animal Sciences, Chair Animal Nutrition, Goettingen, Germany

Corresponding author: Susanne.velten@agr.uni-goettingen.de

Currently, alternative protein sources such as processed insect meals are in special focus of animal nutrition as means to replace imported feed proteins like soybean meal (SBM). As a part of the multidisciplinary project “sustainability transitions” this study aimed to evaluate the potential of *Hermetia illucens* larvae meal (HIM) in meat type chicken diets with complete substitution of SBM during the whole growth period. In total, 210 one-day-old male chickens (Ross 308) from a commercial hatchery were randomly allotted to three diets at feed supply on free choice level. The control diet 1 (main ingredients: SBM, wheat, corn) contained 39% SBM (starter period, d1-21) or 33% SBM (grower period, d22-34). In diet 2, 100% of SBM was replaced by the HIM under study. Both diets were amino acid (AA) supplemented according to current ideal AA ratio (IAAR) recommendations (1). In t diet 3, the content of the first limiting AA (LAA) as calculated (Scontaining AAs) was reduced to 80% of the recommended supply, both for demonstrating the importance of adequate supplementation and for further evaluation of the individual dietary AA efficiency according to the ‘Goettingen approach’. Zootechnical response was under weekly control (Growth, feed intake, feed efficiency, protein conversion ratio, mortality). In addition, several physiological parameters were measured at the end of the growth study (Nutrient deposition, dietary protein quality, apparent precaecal digestibility, epithelial microstructure of the small intestine, selected microbial parameters in gut contents). Based on some of the results, the feed potential of processed insect meal from *Hermetia illucens* in chicken diets will be discussed.

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Extrusion texturization of cricket flour (*Acheta domestiscus*) and soy protein isolate: influence of insect content, extrusion temperature and moisture level on extruder response and texture properties

Anna Martin[1], Samuel Kiiru[2], Raffael Osen[1], John N. Kinyuru[3], Anna-Kristina Marel[2]

[1] *Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany*
[2] *Max-Rubner-Institut Federal Research Institute of Nutrition and Food, Food Technology and Bioprocess Engineering, Karlsruhe, Germany*
[3] *Jomo Kenyatta Univesity of Agriculture and Technology, Food Science and Technology, Nairobi, Kenya*

Corresponding author: anna.martin@ivv.fraunhofer.de

Due to the increasing global population, the demand for further sustainable protein sources ascends constantly. Therefore, insects have become interesting as food ingredients, since they contain valuable amounts of protein and fat, have a comparatively low negative impact on the environment and the area required for cultivation is rather small. One possibility to include insects in the human diet is in the form of meat analogues that are produced using extrusion cooking. A potential species for the application in food is cricket (*Acheta domesticus*).

As cricket flour has only scarcely been investigated as biomass for meat substitutes, the aim of this research was to explore the potential use of cricket flour as protein-rich ingredient for the development of high quality extruded products. For that purpose, the impact of cricket inclusion levels, extruder barrel temperature and moisture content on extrusion system parameters and product properties (e.g. texture) were systematically evaluated.

Cricket flour levels of 15, 30 and 45 g/100g (defatted and undefatted) were combined with soy protein isolate and extruded on a laboratory co-rotating twin-screw extruder with a throughput of 1 kg/h at 150 rpm screw speed. Cooking temperature (120-160°C) and moisture level was varied. Texture was evaluated by texture profile analysis and fiber formation was monitored by assessment of the cutting strength of extrudates. The amount of added cricket flour as well as extrusion temperature had significant effects on texture properties and fiber formation in extrudates.

Continuous production of antimicrobial peptides in insect cells

Lukas Paul Käßer, Tobias Weidner, Denise Salzig, Peter Czermak

TH Mittelhessen University of Applied Sciences (THM), Institute of Bioprocess Engineering and Pharmaceutical Technology (IBPT), Giessen, Germany

Corresponding author: lukas.kaesser@lse.thm.de

Since the 1950s pathogenic microorganisms develop resistances to multiple classes of antibiotics. Treatment of some of these strains with available antibiotics is difficult and the development of new antibiotics stagnates since the late 1990s. Therefore new, effective processes for the production of novel active substances such as antimicrobial peptides (AMPs) are required. Here we demonstrate the use of stably transformed *Spodoptera frugiperda* (Sf9) cells for the production of sarcotoxin, an AMP derived from *Lucilia sericata*.

The research project comprises the establishment and intensification of a platform process for the continuous production of AMPs in insect cells. Key factors currently limiting the productivity are the low cell density in batch or fed-batch cultivation and the lytic nature of the commonly used baculovirus vector expression system. To resolve these bottlenecks, a continuous perfusion process for the cultivation of stably transformed monoclonal insect cells provides a potential solution.

To cultivate the semi-adherent host cells in suspension, a bioreactor setup equipped with modules for perfusion and oxygenation of the culture medium will be developed, and appropriate process strategies will be examined. The complexity of biotechnological production requires not only online-analysis of physical parameters but also information about the bioprocess itself. Therefore online-sensors for biomass will be integrated in the reactor setup to understand, control and design the manufacturing process. Key steps of the project are single cell cloning, process intensification, medium adaption and AMP activity testing.

Bioactive substances from *Hermetia illucens* larvae

Ariane Müller[1], Diana Wolf[2], Katharina Starke[1], Herwig O. Gutzeit[1]

[1] *Technische Universität Dresden, Fakultät Biologie, Institut für Zoologie und Entwicklungsbiologie, Dresden, Germany*
[2] *Technische Universität Dresden, Institut für Mikrobiologie, Professur für Allgemeine Mikrobiologie, Dresden, Germany*

Corresponding author: ariane.adam@tu-dresden.de

The larvae of the black soldier fly, *H. illucens*, feed on a variety of decomposing organic substrates which are typically inhabited by a range of different microorganisms like bacteria and fungi. Survival in these habitats requires a powerful defense against microorganisms and hence it can be expected that the *H. illucens* larvae strongly express AMPs and other substances possessing antibiotic activity. It is well known that insects have a well-developed innate immune system, subdivided into cellular and humoral defense responses. The latter involve the production of AMPs that are synthesized in the fat body and subsequently secreted into the hemolymph. In general, AMPs can serve as effective antibiotics or fungicides that mainly attack the cell envelope, especially the cell membrane and also affect intracellular targets of microorganisms which subsequently lead to cell death. Several recent studies that reported the antimicrobial activity of the larvae hemolymph and maggot extract as well as of secretions, are promising for the development of new therapeutically valuable antibiotics, particularly in the defense of multi-resistant “super bugs” (e.g. the ESKAPE strains Staphylococcus aureus or Pseudomonas aeruginosa). In our lab, we use extracts and hemolymph of immunized *Hermetia illucens* larvae to study the bioactive effects on biofilms of several Gram-positive and Gram-negative microorganisms and to analyze the mode of action of *H. illucens* larvae AMPs on the Gram-positive model organism Bacillus subtilis in more detail. Further experiments regarding the purification of the crude larval extracts by chromatography as well as sequencing and structure determination of produced peptides are currently in progress.

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Volatile compounds emitted during Black Soldier Fly larvae rearing, show antagonistic effect toward molds

Doron Levy[1], Chen Katz[2], Adi Jonas-Levi[3]

[1] *Biotechnology Department, Faculty of Sciences and Technology, Tel Hai College, Qiryat Shemona, Israel*
[2] *Molecular and Environmental Microbiology Laboratory, MIGAL – Galilee Research Institute LTD, Kiriat Shmona, Israel*
[3] *Food Sciences Department, Faculty of Sciences and Technology, Tel Hai College, Qiryat Shemona, Israel*

Corresponding author: adijon@gmail.com

Black soldier fly (*Hermetia illucens*, BSF) is a relatively big fly originating from the tropic and subtropics areas of Latin America and now is spread throughout many other regions of the world. Its larvae (BSFL) are well-studied as a possible source of proteins for food and feed as well as lipids for biodiesel production. The larvae have the capability to process large quantities of organic matter and reduce its volume by 60% while bioconverting it to body mass. Thus, waste processing by BSFL Is a potential tool for making organic waste into valuable products. Here we report that BSFL rearing is accompanied by the production of mold inhibiting volatiles such as 2-Heptanon, Dimethyl disulfide and Acetic acid that prevent germination of mold spores. We have studied the contribution of several growth conditions to this phenomenon and show that the larval stage and rearing temperature are key factors in mold inhibition.

Utilization of Yarrowia-like strains isolated from *Nicrophorus vespilloides* as platform for intended production of active protein aggregates

Oliver Birrenbach[1], Doreen Gerlach[2], Peter Czermak[1]

[1] *Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Giessen, Germany*
[2] *Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Department of Bioresources, Giessen, Germany*

Corresponding author: oliver.birrenbach@ime.fraunhofer.de

A considerable number of different microorganisms is present in the anal secrets of the burying beetle (*Nicrophorus vespilloides*). To investigate the microflora that is able to digest carrion and defend it against competitors, several organisms have been isolated and determined. In this work Yarrowia-like yeasts, from this microflora, are used to develop a platform for recombinant protein production of biological active aggregates. The isolated yeast strains show great potential for biotechnological use due to their genomic similarity to the industrial important yeast Yarrowia lipolytica. For a long time insoluble aggregates of proteins have been seen as an unwanted by-product of recombinant protein production. However aggregates possess properties that are favourable for recombinant proteins. As aggregates enhance the stability against environmental influences like pH, temperature and protect the protein against proteases. The challenge is to contain the activity despite the aggregation. Therefore an intended and controlled production of proteins as biological active aggregates by Pull-Down-Tags (PDTs) reveals a suitable method to simplify up- and downstream processes. Driving proteins into an insoluble production of aggregates, PDTs are investigated as fusion proteins for their pull down efficacy and the remaining activity of the Protein of Interest (POI). A combinatory library of PDTs and POIs is prepared and screened by FACS for a multiple integration into Yarrowia-like strains genome. Enzymes as well as antimicrobial peptides are produced and analysed for their biological activity. Forming nanoparticle-like aggregates this quasi-immobilized enzyme production is designed for packed-bed reactor application.

Testing three artificial light sources on oviposition and half-life of Black Soldier Fly to improve small-scale indoor rearing

Carina Desirée Heussler[1], Andreas Walter[2], Hannes Oberkofler[3], Heribert Insam[3], Wolfgang Arthofer[4], Birgit C. Schlick-Steiner[4], Florian M. Steiner[4]

[1] *University of Innsbruck, Ecology, Mikrobiology, Innsbruck, Austria*
[2] *Management Center Innsbruck, Biotechnology & Food Engineering, 6020 Innsbruck, Austria*
[3] *University of Innsbruck, Microbiology, Innsbruck, Austria*
[4] *University of Innsbruck, Ecology, Innsbruck, Austria*

Corresponding author: Carina.Heussler@uibk.ac.at

As human population is expanding, waste management is becoming more challenging. A new approach here is composting using the digestion by insect larvae. The Black Soldier Fly (*Hermetia illucens*) is interesting for biodegradable waste management as it can reduce the amount of organic waste and produce economically relevant quantities of larval feedstuff for livestock. The flies occur naturally in (sub)-tropical and warm-temperate climates, and their mating depends on space and sunlight. To make use of the Black Soldier Fly in cold-tempered regions, a small-scaled indoor rearing system is necessary, with controllable temperature and humidity (27°C and 60%, respectively) and artificial light. Recent studies have shown that small-scale indoor rearing (cage size: 27 × 27 × 27 cm) under artificial light is feasible. In this study, we compare the influence of three artificial light sources (light-emitting diodes, fluorescent and halogen lamps) for small-scale indoor rearing. Three experiments were conducted to compare oviposition traits (pre-oviposition period, total oviposition-period, and egg mass per female) and half-life. We found that oviposition in the cages we used (29 × 28 × 39 cm) worked fine and did not differ significantly. However, the halogen lamps resulted in a significantly shorter half-life of males and females than light-emitting diodes and fluorescent lamps in all experiments. Based on the performance of the light-emitting diodes and their outstanding energy efficiency, we recommend this light source for small-scale indoor rearing of the Black Soldier Fly.

Influence of corn by-products on chemical composition of Black Soldier Fly (*Hermetia illucens*) larvae

Marco Meneguz[1], Andrea Dama[1], Christian Caimi[1], Marta Gariglio[2], Achille Schiavone[2], Francesco Gai[3], Laura Gasco[1]

[1] *Università degli Studi di Torino, Department of Agricultural, Forestry and Food Sciences, Grugliasco, Italy*
[2] *Università degli Studi di Torino, Department of Veterinary Science, Grugliasco, Italy*
[3] *National Research Council, Institute of Science of Food Production, Grugliasco, Italy*

Corresponding author: marco.meneguz@unito.it

The world corn production is estimated in 1Mton in 2016 (FAOSTAT 2016). The production of corn get use as feed and food, while corn by-products are usually wasted or used as combustible. Stover corn (SC) obtained by corn cutting is an economic low-value material high in fiber, with a low protein (3-5%) and sucrose (0.6-1.2%) content (HOSKINSON *et al.* 2006). BSF is a promising species for feed production (HENRY *et al.* 2013, SCHIAVONE *et al.* 2018, GASCO *et al.* 2018) and waste management (DIENER *et al.* 2011) and it is adapted to grow in different kinds of organic matter (SPRANGHERS *et al.* 2017, MENEGUZ *et al.* 2018). The aim of this research was to evaluate (SC) as feed for black soldier fly (BSF) larvae. Gainesville diet (50% wheat bran, 30% alfa-alfa meal and 20% corn meal) (HOGSETTE 1992) was used as control (SC0), moisture 70%, and compared to three experimental diets where 25%, 50% and 100% of SC0 was substituted by SC (SC25, SC50, and SC100, respectively). 500 larvae (5 days-old) were counted per replicate (4 replicates/treatment) and placed in plastic container (25 x 33 x 15 cm), randomly allocated in a climate chamber with 28°C T and 70% RH. Data were analyzed by IMB SPSS software for Windows, using a One way-ANOVA and Tukey's post hoc test was used to compare the pairs, with a significance $p \leq 0.05$. Concerning substrates, preliminary results showed as the crude protein (CP) decreased from SC0 (15.16±0.04%) to SC100 (5.88±0.19%), while fiber fractions (NDF, ADF and ADL) showed an opposite trend. The highest ether extract (EE) content was highlighted in SC0 (2.53%).

SC100 larvae highlighted difficulties in growth and did not reach the prepupae stadium. They were thus not considered for analyses. No differences were reported for dry matter (DM) (29.9±1.09; 29.6±0.56), CP (11.0±0.65%; 12.8±0.77%), ADF (7.5±0.80%; 7.2±0.40%) and chitin (4.1±0.96%; 4.0±0.37%) between larvae fed SC0 and SC25. Larvae fed SC50 showed the highest ($p < 0.05$) content of chitin (5.8±0.82%) while the highest values of EE (14.5±0.95%) was recorded in SC0. Preliminary results showed how low inclusion (SC25) of SC does not negatively affect the larvae CP content and could represent an interesting low-cost substrate in BSF production. SC50 seemed to be interesting for chitin production, while it showed lower CP and EE values than SC0 and SC25. Next researches has to evaluate inclusion of SC in industrial diet for BSF mass rearing production and tolerance of BSF larvae on insecticides that could be present in SC.

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Israel national center for the expression of beneficial insects

Yoram Yerushalmi

Israel National Center for the Expression of Beneficial Insects, Afula, Isarel

Corresponding author: yoram.yerushalmi@gmail.com

The ever-growing interest in insect farming and the use of beneficial insects, both in agriculture and food, as well as an extremely potent environmental solution for organic waste management, led us to the decision to open the worlds' first center for the expression of beneficial insects in Afula, Israel. The center is aiming at serving as a knowledge hub for diverse sectors such as the industry, academia, agriculture, Health, other governmental offices, and last but not least, the general public. Nonetheless, the main goal and purpose of the center is to host entrepreneurship leadership and multiple startups working and focusing on different aspects of insects led solutions, contributing to our many aspects and growing needs of modern life. We aim to develop a work frame which will recruit and host promising startups and entrepreneurs from all over, to come together and work in an insect centered environment in the proliferate historically green spot of Israel, Yizrael valley. I will describe the structure and current status of the center, as well as present the call for the first class of startups.

Processing of agricultural plant waste using the Black Soldier Fly larvae causes germination reduction in various weed types

Adi Jonas-Levi, Jean-Jacques Itzhak Martinez, Ofer Danay, Liora Shaltiel-Harpaz

Tel-Hai College, Upper Galilee, Kiryat Shmona, Israel

Corresponding author: adijon@gmail.com

In Israel, huge amounts of agricultural plant wastes are produced annually. Most of it is handled in an environmentally inconsiderate way, being left in the fields and thus becoming a source of environmental nuisance, including weed propagation. In a study aimed to provide an economic incentive for farmers to collect and treat plant residuals, we investigated the use of the black soldier fly (BSF), *Hermetia illucens* (L.), (*Diptera: Stratiomyidae*). The BSF larvae consume large quantities of organic matter. Two valuable products obtain from the process, protein for animal feed and organic residues. In order to use these residues as fertilizer, they must be cleansed from harmful factors such as weed seeds.

In this study we investigated the ability of the BSF larvae to reduce germination of three types of weeds, *Orobanche aegyptiaca* Pers., *Chenopodium album* and *Setaria verticillata* as models for absolute parasites, broadleaf weeds and cereal grass weeds, respectively. The weed seeds were introduced in a controlled manner to the substrate and in the atmosphere of BSF larvae rearing facility. The results indicated that BSF larvae significantly reduced weeds germination by 98.9%, 100% and 99.7% respectively, through three mechanisms: digestion and destruction of the seeds, phytotoxicity of the substrate and secretion of volatile seed germination inhibitors. We conclude that in addition to the direct economic gain from BSF larvae growth on agricultural plant waste, an environmental gain may also be added due to the significantly reduction of weeds, and as a result a decrease need for herbicides.

Apparent digestibility of insect-based PAPs for rainbow trout feeds

Laura Gasco[1], Christian Caimi[1], Achille Schiavone[2], Giuseppe Serra[3], Roberta Anedda[4], Angela Trocino[5], Marta Gariglio[2], Ilaria Biasatoio[2], Carola Lussiana[1], Marco Meneguz[1], Christophe Trespeuch[6], Francesco Gai[3]

[1] *University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, Italy*
[2] *University of Turin, Department of Veterinary Sciences, Grugliasco, Italy*
[3] *National Research Council, Institute of Ecosystem Study, Grugliasco, Italy*
[4] *Porto Conte RicercheTramariglio, Alghero SS, Italy*
[5] *University of Padova, Padova PD, Italy*
[6] *Mutatec, Châteaurenard, France*

Corresponding author: laura.gasco@unito.it

Aquaculture is one of the fast growing food producing sectors accounting for nearly the 50% of the fish production in 2016 (FAO, 2016).

Fishmeal has been the main processed animal protein (PAP) ingredient used in aqua feed but nowadays, due to both the limited available quantities and the increase in demand, its inclusion is reduced to a minimum amount (OLIVA-TELES *et al.* 2015, GASCO *et al.* 2018). Other PAPs are used such as poultry by-product meal, meat and bone meal or feather meal as well as several plant protein meals (soybean meal, gluten meals, soy protein concentrate). Insect-based PAPs have attracted much attention (HENRY *et al.* 2015, GASCO *et al.* 2018) especially for their use in carnivorous species that hardly face limitation due to antinutritional factors contained in plant proteins. The European Commission has recently approved the use of PAPs from seven insect species in aquaculture (Reg. 2017/893/EC) and, consequently, their use in aquafeed is expected to increase. Digestibility is the quantification of the digestive process and provides information on the extent to which an ingested aliment and its nutrient components have been digested and retained by the fish (BUREAU *et al.* 1999). The correct knowledge of an ingredient digestibility is fundamental to assess its potential for inclusion in fish diets for the optimization of both its economic and environmental sustainability. Apparent Digestibility Coefficient (ADC) provides good indication of the nutrients bioavailability of and energy, thus providing rational basis for the correct inclusion of feedstuffs. However, due to the lack of data, insect-based PAPs have so far been included in fish diets on the basis of their nutrients content and energy density without taking into account their biological availability

Therefore, the purpose of this study was to assess the ADCs of four defatted insect-based PAPs for rainbow trout: two *Tenebrio molitor* (TM1 and TM2) obtained from two different producers, one *Hermetia illucens* (HI), and one *Alphitobius diaperinus* (AD).

A total of 180 rainbow trout were stocked into twelve 250^{-L} digestibility tanks with 15 fish per tank. Each tank was connected to an open water system (artesian well water with constant temperature of 13 ± 1°C). Following the protocol recommended by BUREAU *et al.* (1999), a high-quality reference diet (R), able to cover the fish requirements,

and five experimental diets, obtained mixing the R diet with each of the insect-based PAP at a ratio of 70:30, were formulated. Diets were added with 1% celite® (Fluka, St. Gallen, Switzerland) as inert marker. Fish were fed to visual satiety twice a day and faeces collected using a continuous automatic device (CHOUBERT *et al.* 1982). Faeces were freeze dried and frozen (-20°C). Insect-based PAPs, experimental diets and faeces were analysed (AOAC INTERNATIONAL 2000, 2003). The insect meal crude protein (CP) content was calculated using the nitrogen to protein ratio proposed by JANSSEN *et al.* (2017). The ADC of dry matter, (ADCDM), CP (ADCCP), and ether extract (ADCEE) were calculated following the equation recommended by BUREAU & HUA (2006). Data were tested by means of one-way ANOVA and significance was declared at $P<0.05$. ADCDM varied between 63.39% (HI and AD) and 91.41% (TM1). AD showed the lowest ADCCP (74.44%) while the highest was presented by TM1 and HI (91.99% and 89.84%, respectively). Concerning ADCEE values reported were above 96% in all treatments. The two different TM showed significant differences for DM and CP digestibility coefficients. Differences reported among insect-based PAPs are imputable to the insect species, but they could also be related to the meal treatment. Tested insect meals resulted highly digestible for rainbow trout and these preliminary results could be useful to optimize the diet formulation.

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Fuhneschrecken: an everyday food?

Thorsten Breitschuh, Cornelia Häffner
Agrar Insekt Anhalt GbR, Werdershausen, Germany
Corresponding author: hallo@jump-food.de

Saxony-Anhalt supports innovative projects under the EU's ELER program.

The aim of the Agrar-Insekt GbR is to go new ways of sustainable, environmentally friendly and future-oriented agricultural production in the project. In addition to the two partners Thorsten Breitschuh and Dr. Ing. Cornelia Häfner, who are dealing with the keeping, propagation, preparation and marketing of insects as key players, is supported by competent experts from Anhalt University of Applied Sciences and ppm Magdeburg e.V. In doing so, three goals of sustainable agriculture are being worked on:

1. Adaptation of agricultural production to climate change through the use of new species
2. Production of a proteinaceous food
3. Utilization alternative of grassland

Edible insects are rich in high-quality protein, so they could act as purely nutritionally physiologically considered a meat alternative. This may not be an argument for "real meat fans", but if one compares the environmental balance of the production of the two protein suppliers, the insects are the clear winners: As cold-blooded animals, they do not have to apply energy to warm the body and have far greater feed conversion efficiency than traditional livestock, Compared with pig farming, they produce 10 to 100 times less harmful gases per kilogram of body mass. Our grasshoppers are fed exclusively by grassland crops grown on the farm.

In view of the growing population and changing dietary habits, which are reflected, among other things, in a global increase in the total consumption of meat, the search for alternatives to the classical animal protein suppliers is urging itself on. Many edible insect species are rich in high-quality protein, followed by fat, fiber and carbohydrates. Grasshoppers can even contain up to 77 percent protein. The fat content of edible insects is on average between 13 and 33 percent dry matter. Their energy value, based on the fresh mass, is comparable to that of meat. With their sometimes high levels of unsaturated fatty acids, edible insects can also compete with some fish species. Grasshoppers are also rich in folic acid.

It is exciting, if edible insects in this country can be admitted as food and then offered. Edible insects are among the so-called novel food products. To this end, a special permit must be requested from the European Food Safety Authority (EFSA) in the project.

Morphological and molecular study of larval and adult *Hermetia illucens* L. (Diptera: Stratiomyidae) olfactory system

Andrea Scala[1], Marisa Nardiello[1], Rosanna Salvia[1], Carmen Scieuzo[1], Antonio Franco[1], Heiko Vogel[2], Patrizia Falabella[1]

[1] *University of Basilicata, Department of Science, Potenza, Italy*
[2] *Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany*

Corresponding author: patrizia.falabella@unibas.it

Insects use chemical perception to interact with other insects and with the environment. The perception of volatile substances, linked to the processes of feeding, mating, escaping from predators and searching for oviposition sites is mediated, among the others, by Odorant Binding Proteins (OBPs). In order to select optimal oviposition sites, adult black soldier flies *Hermetia illucens* L. (Diptera: Stratiomyidae) are attracted to molecules deriving from organic material. Similarly, the larval stages of the black soldier fly have a highly sensitive olfactory system, able to perceive molecules coming from organic substrates. To identify chemoreception candidate transcripts, RNA-seq was performed on *Hermetia illucens* antennae and whole body of male and female adults as well as on *H. illucens* whole larvae. The *de novo* transcriptome assembly resulted in 78,763 contigs in adult transcriptomes and 25,197 contigs in larval transcriptome. Functional annotation using Blast2GO software and following analysis identified 60 putative OBPs in adult transcriptomes and 18 putative OBPs in larval transcriptome. All the 18 larval OBPs were compared with all the 60 adult OBPs and a high level of similarity, and in some cases a perfect identity, was found in terms of aminoacid sequence. This result confirms that OBPs play a key role in *Hermetia illucens* olfactory perception. Moreover, the identification of volatile organic compounds (VOCs) emitted by different substrates colonized with black soldier fly larvae was conducted. A parallel investigation by scanning electron microscopy (SEM) was performed on *Hermetia illucens* larvae and adults to identify structures possibly related to the olfactory perception.

Insect Odorant Binding proteins are promising candidates for the development of innovative biosensors

Rosanna Salvia[1], Andrea Scala[1], Donatella Farina[1], Antonio Moretta[1], Heiko Vogel[2], Patrizia Falabella[1]

[1] *University of Basilicata, Department of Science, Potenza, Italy*
[2] *Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany*

Corresponding author: patrizia.falabella@unibas.it

The emission and the perception of chemical molecules represent the main communication system for insects as it regulates vital functions such as the selection of food substrates, mating, selection of oviposition sites and dangers avoidance. The perception of volatile substances is related to gene families encoding for proteins belonging to Odorant Binding Proteins (OBPs), Olfactory Receptors (ORs), Ionotropic Receptors (IRs) and Chemosensory Proteins (CSPs). Specifically, OBPs are able to bind the hydrophobic chemical molecules allowing them to cross the sensory lymph and bind to the olfactory receptors on the dendrites of sensory neurons, activating the signal transduction processes. The variety of insect OBPs and their involvement in smelling volatiles of different nature suggest the use of these proteins in a multiplicity of biotechnological applications. Insect OBPs can be used as biological sensory unit for the development of biosensors to detect Volatile Organic Compounds (VOCs), present in food and beverages. The characterization of the aroma of wines represents an interesting challenge. Grape and wine terpenes play a fundamental role in the definition of quality and typicity. Different aphid OBPs are known to mediate the perception of some terpenes e.g. farnesol, geranyl acetate and limonene. In other cases, undesired compounds can negatively affect the organoleptic quality of wines, adding unpleasant aromatic and taste characteristics. Among undesired compounds, ethyl phenyl acetate is a pheromone of different species of Hymenoptera whose OBPs will be identified and characterized.

Transcriptomic and proteomic approach for the identification of *Torymus sinensis* (Hymenoptera: Torymidae) venom gland proteins

Rosanna Salvia[1], Andrea Scala[1], Antonio Moretta[1], Donatella Farina[1], Piero Pucci[2], Alberto Alma[3], Chiara Ferracini[3], Heiko Vogel[4], Patrizia Falabella[1]

[1] *University of Basilicata, Department of Science, Potenza, Italy*
[2] *University of Naples Federico II, Department of Chemical Sciences, Naples, Italy*
[3] *University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, Italy*
[4] *Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany*

Corresponding author: patrizia.falabella@unibas.it

Torymus sinensis Kamijo (Hymenoptera: Torymidae) is a hymenopteran wasp used for the biological control of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), a globally invasive pest of chestnut, *Castanea* spp. *T. sinensis* is a univoltine ectoparasitoid, sometimes exhibiting a prolonged diapause, with a life cycle synchronized with its host. It is the dominant parasitoid species reared from *D. kuriphilus* galls in its native China and has been introduced into many countries of Asia, North America, and Europe for the gall wasp populations management. We used high-throughput nucleic acid sequencing methods to describe the transcriptome of *T. sinesis* venom gland. From *de novo* assembly of all cDNA, 22,874 contigs were obtained and all the sequences were analysed by Blast2GO software for the functional annotation and for the analysis of genes or protein sequences. An overall picture of putative proteins present in the venom gland was provided by transcriptomic information which also gave information on molecular functions and biological processes. Venom components were also analysed by proteomic methodologies and fractionated by SDS-PAGE electrophoresis. Protein bands were excised from the gel and, after tryptic digestion, were identified by mass spectrometry (MALDI TOF/TOF and LC-MS/MS). The comparison between the protein sequences identified by the MaxQuant software and the transcriptomic data, allowed the identification of numerous putative proteins of *T. sinensis* venom. Molecular identification and subsequent characterization of these molecules will allow to understand the role played by venom in the induction and regulation of the pathological syndrome observed in parasitized hosts.

Toxoneuron nigriceps (Hymenoptera:Braconidae) teratocytes: generation and analysis of a comprehensive transcriptome for the identification of antimicrobial peptides

Rosanna Salvia[1], Andrea Scala[1], Antonio Moretta[1], Carmen Scieuzo[1], Marisa Nardiello[1], Donatella Farina[1], Heiko Vogel[2], Patrizia Falabella[1]

[1] *University of Basilicata, Department of Science, Potenza, Italy*
[2] *Max Planck Institute for Chemical Ecology, Department of Entomology, Jena, Germany*

Corresponding author: patrizia.falabella@unibas.it

Insect host-parasitoid systems are examples of complex interactions. Parasitoids have fit to their respective hosts by developingseveral strategies ensuring the success of parasitism, providing physiological alterations and overcoming the immune response of their host. A key role in host regulation and immune suppression is played by maternal factors introduced by the female wasps into the host during egg deposition together with embryonic factor. Teratocytes are specialized cells deriving from the dissociation of the membrane surrounding the parasitoid embryo (serosa), released in the host haemocoel when the parasitoid egg hatches. To date, only a putative chitinase has been identified in *Toxoneuron nigriceps* (Hymenoptera:Braconidae) teratocytes. A *de novo T. nigriceps* teratocytes transcriptome has been generated by high-throughput RNA sequencing (RNA-Seq). Data assembly resulted in 24,569 contigs which were annotated by BLASTx algorithm against the nr database and functionally analysed byBlast2GO software. The distribution of the species associated with top BLAST hitsshowed that a high proportion of genes in the *T. nigriceps* teratocyte transcriptome was most similar to genes belonging to different parasitoid wasp species, in particular to *Microplitis demolitor.* Putative antimicrobial peptides (AMPs) were identified and will be produced and functionally characterized. In add, ovalbumin-related X-like proteins, serpin proteins, chitinases and Rho GAP proteins, potentially involved in host immunosuppression, were identified. These preliminary results led us to hypothesize that teratocytes are not only responsible for host immunosuppression but at the same time could prevent host infection by pathogen intruders, supporting the immunosuppressed host through the production of parasitoid-derived AMPs.

Digestibility of different dietary fibres by Black Soldier Fly larvae

Moritz Gold[1,2], Robert Spiess[1], Christian Zurbrügg[2], Michael Kreuzer[3], Alexander Mathys[1]

[1] ETH Zurich, Institute of Food, Nutrition and Health, Sustainable Food Processing Laboratory, Zurich, Switzerland
[2] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Sandec: Department of Water, Sanitation and Solid Waste for Development, Dübendorf, Switzerland
[3] ETH Zurich, Institute of Agricultural Sciences, Animal Nutrition, Zurich, Switzerland

Corresponding author: moritz.gold@hest.ethz.ch

Introduction

Processing of biowaste by black soldier fly larvae (BSFL), *Hermetia illucens* L. (Diptera: Stratiomyidae), is a relatively new treatment technology that has received considerable attention in the last years (ČIČKOVÁ *et al*. 2015, DORTMANS *et al.* 2017). BSFL biowaste processing uses the natural appetite of the larvae for animal manures, human excreta, fruit and vegetable wastes, and carrion (ROZKOSNY 1983, SCHREMMER 1986). When feeding on biowaste, in addition to a residue similar to compost, this technology produces larval biomass that is high in fat and protein (each around 30-40% of dry larval mass) and therefore has a high market value as animal feed (fish meal often used as a reference is valued at around 1600 USD/ton, based on data cited by (FAO 2016) (MAKKAR *et al.* 2014, WANG & SHELOMI 2017). High amounts of lauric acid, antimicrobial peptides, and chitin in BSFL are other potential benefits for its use as an animal feed (GASCO *et al.* 2018). Revenues from BSFL and the residue can foster the implementation and operation of biowaste management systems that are financially sustainable, especially in fast-growing developing countries (DIENER *et al*. 2011, DORTMANS *et al*. 2018). BSFL processing can also produce larval biomass that, if used as feed, can have a lower environmental impact than conventional feed (SMETANA *et al*. 2016).

One challenge for the large-scale implementation of BSFL biowaste processing is the variable process performance (e.g. larval yield) on different types of biowaste (e.g. vegetable and fruit waste, municipal organic solid waste, cow manure). This can jeopardize a sustainable and economic operation. A key driver for this variable process performance are thought to be variable quantities and qualities of macronutrients (i.e. protein, carbohydrates, fibres, lipids).

In controlled feeding experiment with chemically defined diets, this research studied the digestibility of seven different dietary fibres in BSFL biowaste processing. The results should inform more accurate biowaste formulation to ensure a stable process performance for efficient BSFL biowaste processing and animal feed production.

Material and methods

BSFL were obtained from a black soldier fly colony (~25 °C, <70% relative humidity, light: 10 hours, dark: 14 hours), maintained at Eawag in Dübendorf, Switzerland. In this research, 4-7 day old larvae with an average weight of 1.3 mg were used. These were reared on commercial chicken feed (UFA AG, Herzogenbuchsee, Switzerland). Larval hatched and developed on the chicken feed in a climate chamber (Memmert, HPP 260, 28°C, 70% relative humidity).

This research formulated seven diets based on CAMMACK & TOMBERLIN (2017). Each diet contained the same amount of protein, carbohydrates, fibres, lipids, vitamins and minerals. Only the fibre composition which accounted for 54% of diet dry mass was varied using the following fibres: α-cellulose (Cellos), β-(1,3;1,4)-glucan (bGl), glucomannan from konjac (Gma), xylan from corn (Xyl), inulin from chicory (Inu), lignin (Lig) and pectin from citrus (Pec). During diet formulation, bGl was combined with Lig, Xyl with Gma, and Inu and Pec with Cellos. Celite (i.e. Diatomaceous earth) which includes no fibre was used as a negative control. Before the start of the experiment, tap water was added to produce diets with a moisture content of 70%.

At the beginning of the experiments, 125 larvae were manually counted and added to the diets that were placed in disposable plastic containers and covered with kitchen tissue. Each diet consisted of three biological replicates. According to CAMMACK & TOMBERLIN (2017), the feeding rate was five gram per day. Feed was provided to the larvae on day 0, 2, 5, 7, 9, and 12. The larvae were kept in a climate chamber during the entire experiment to secure stable environmental conditions (Memmert, HPP 260, 28°C, 70% relative humidity). The experimental duration was 14 days.

Parameters that we determined in this research included feed and residue soluble (SDF) and insoluble fibre (IDF), larval weight, survival rate, bioconversion rate and waste reduction. Soluble and insoluble fibre of the diets and residue were analysed enzymatically using a commercial kit (MEGAZYME 2016). The other parameters were determined gravimetrically using a precision balance according to REHMAN *et al*. (2017).

Statistical analyses were performed in Rstudio 1.1.442. Significant differences between mean results of the three biowaste treatments were identified using one-way analysis of variance (Anova), followed by the post-hoc Tukey HSD (honestly significant differences) test. A p-value < 0.05 was considered as a measure of statistical significance. The authors assumed that the data of each parameter followed a normal distribution.

Results

Larvae developed well on all diets indicated by a survival rate of >90%. However, the Lig/bGl and Xyl/Gma diets had very different textures than all other diets which likely influenced identification of dietary fibre digestibly by BFSL on these diets. The Lig/bGl diet had a sticky texture. This could have reduced movement and feeding of the larvae and explain the significantly lower larval weight, waste reduction and bioconversion rate on this diet in comparison to all other diets. Xyl/Gma had a rubber-like texture.

This prevented the homogeneous distribution of the diet and led to diet clumps with mould. Therefore, these diets were excluded from further analyses.

Waste reduction is an indication of fibre digestibility when it exceeded the 54% fibre content of the diets used in this study. Pec had a waste reduction > 54% which was significantly higher than those of Inu, Pec and Cellos. This indicates that Pec was digested by BSFL. In contrast, Cellos had a significantly lower waste reduction than Inu, Pec and Celite, indicating that it has a low or no digestibility by BSFL.

Mass balance of dietary fibre provided at the beginning of the experiment and the residue at the end of the experiment confirm the results for waste reduction. Considering the accuracy of the commercial dietary fibre kit, cellulose was not digestible for BSFL. In contrast, the SDF in Inu and Pec was reduced by >70%. As the SDF in these diets is exclusively comprised of inulin from chicory and pectin from citrus, these results suggest that the two sources of dietary fibre are digestible by BSFL and/or its associated gut microbes. Interestingly, IDF in the Pec was also reduced. As pectin contained no IDF, this suggests that some cellulose was digested when cellulose was combined with pectin.

As biowaste typically consists of <5% SDF (pectin and inulin are both soluble fibres) and most fibre in biowaste are IDF (such as cellulose), a conservative approach for diet formulation would be to consider dietary fibre as having no direct nutritional value. In this study, the process performance was significantly higher when all cellulose was replaced by Celite. However, as indicated by previous research, IDF may be decomposed by microbes in the biowaste or BSFL gut (REHMAN *et al*. 2017, ZHENG *et al*. 2012).

Conclusions

Variable process performance of BSFL on different types of biowaste is a challenge of this technology for biowaste management and economic and sustainable animal feed production. Different quantities and qualities of macronutrients, such as dietary fibre, are likely one reason for this variability.

This research indicates that inulin and pectin are digestible by BSFL. In contrast, cellulose, the most common dietary fibre in biowaste, is not digestible. Future research should repeat these experiments with an emphasis on minimizing the influence of diet texture and microbiology. Understanding the digestibility of dietary fibres by BSFL can be used to optimize diet formulation for more stable and efficient biowaste treatment and animal feed production.

Evaluation of a potential dry processing route for transforming insects into food and feedstuff

Sara Bußler, Julia Durek, Antje Fröhling, Oliver Schlüter

Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany

Corresponding author: sbussler@atb-potsdam.de

Utilization of insects for food and feed requires a systematic approach in the area of bioeconomic production. In order to deliver safe and high-quality raw materials and ingredients for food and feed applications the processing of insects is a major prerequisite. The impact of processing methods on the nutritional or functional quality of insects and their components in food such as the protein quality needs to be considered and determined, whereas feasibility and economic aspects of processing steps and processing routes need to be assessed.

Objective of the study was the evaluation of potential processing routes for transforming insects into foodstuff. Exemplary insects used were mealworm (*Tenebrio molitor)* larvae. Along a dry processing route, the insects were inactivated by freezing or boiling, dried, then mechanically defatted (concomitant lipid separation) and ground to an insect meal. This process is already commercially applied for the black soldier fly larvae. The drying process, moreover the impact of the drying temperature on insect quality parameters (protein yield, product colour, water and fat binding capacities) and on the microbial load was investigated in detail. Results confirm that defining the optimum drying temperature is a balancing act considering microbial safety and maximum protein yield. While the protein yield is optimized at drying temperature of 40-60°C, a drying temperature above 120°C is recommended for a microbiologically safe product. It was further found that a certain amount of water present in the pressing step seem to be essential for a satisfactory lipid extraction.

Influence of drying on the recovery of different biopolymers from cricket (*Acheta domesticus*) flour

Sara Bußler, Julia Durek, Antje Fröhling, Oliver Schlüter

Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany

Corresponding author: sbussler@atb-potsdam.de

With respect to the growth of human population and the limited resources for food and feed production, the role of insects for human consumption has lately increased in interest. The production of defined insect fractions is a pre-requisite for the development of standardized food with optimized formulations. As the insect composition greatly varies depending on the insect species, the feeding and the stage of development, in a first approach an evaluation of the specific processing impact on quality characteristics of insect fractions from different developmental stages is needed to derive appropriate processing concepts.

The aim of the study was to develop a process for preparing a protein-rich insect meal from adult and subadult crickets (*A. domesticus*) considering essential process steps as inactivation, milling and drying. Since drying strongly influences product quality, nutritional properties and, to a certain extent, microbial safety, it is the key operation for the production of insect flours. Therefore, the effects of different drying temperatures on major components of insect meal from individual fractions were examined in detail.

For subadult crickets a desired final moisture content of < 5% was reached following 24 h (60°C), 1.5 h (80°C), 75 min (100°C), 50 min (120°C), and 30 min (140°C), whereas it took 21 h (60°C), 2.5 h (80°C), 1.5 h (100°C), and 1 h (120 and 140°C) in case of drying of adult crickets. Protein solubility and flour colour were severely changed depending on the drying temperature.

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Leibniz-Institut für Agrartechnik und Bioökonomie e.V.
Max-Eyth-Allee 100
14469 Potsdam

Tel.: (0331) 5699-820
Fax.: (0331) 5699-849
E-Mail: atb@atb-potsdam.de

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