





## The 2<sup>nd</sup> International Conference 'Insects to Feed the World' (IFW 2018)

15-18 May 2018

Wuhan, China P.R.

This supplement of Journal of Insects as Food and Feed can be found at

https://www.WageningenAcademic.com/IFW2018.

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## Insects for food, feed and health: a global perspective

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During the last ten years there has been a growing interest worldwide to use insects as food, feed or pharma. This is exemplified by the increasing number of start-ups, the emergence of some large international companies, the willingness to invest in this sector, the legislation becoming gradually more conducive and the growing public and academic interest. This is because, it is realized that the growing demand for animal-based protein cannot be satisfied by the amount of agricultural land available. Besides livestock production has a negative impact on the environment, while there are concerns around human health. Insects both as food and feed pose a viable and sustainable alternative, while contributing to a circular economy by transforming low value organic side streams into high value protein products. However, it requires the development of new value chains, and attention to issues such as production costs, food safety, scalability and consumer acceptance. The feed industry in particular requires reliable large supplies of high and standard quality. At the production unit major challenges are: to obtain and maintain a genetic stock; to prevent and control pests and diseases; to choose an appropriate facility design and technology to control temperature, humidity and light; using leftover substrates as fertilizer; and addressing insect welfare. The organic side-stream chosen as feed is of crucial importance as it influences costs, larval development, and the nutritional and food safety quality of the end product. Most edible insect species appear to be good sources of amino acids, fatty acids, and micronutrients. However, there are compounds such as chitin and anti-microbial peptides that may have additional health benefits compared to conventional food and feed sources. Processing can be a simple drying and grinding of the insects or more elaborate biorefinery processes to isolate chitin, protein and fat. Consumers may be persuaded by concentrating on tastiness, product design, marketing and promotion. The legal environment to produce and market insect products is often not conducive and needs major attention. The sector faces huge challenges and needs pro-active support by regional, and (inter)national authorities in terms of legislation, research and training. Large companies are often very secretive about their operations but what about farmers who want to shift from livestock to mini-livestock? The increasing number of stakeholder conferences, symposia and workshops organized at the national and international level shows that a new sector is emerging. It can only be successful if the challenges are met when private, public and academic institutions join forces.

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### The resource of edible insects in China

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Edible insects have attracted extensive attention worldwide over the recent years since they have the potential to resolve the global food shortage and insufficiency of animal proteins. Many countries have a history of using insects as food. It is believed that insects have been used as a food source in China for more than two thousand years. According to incomplete statistics, there are more than 300 insect species which could be used as food or as food ingredient. Most insects are in the orders of Lepidoptera, Coleoptera and Hymenoptera, contributing to 71% of total numbers. Others are insect species in the orders of Orthoptera, Hemiptera, Isoptera, and Odonata. Bees, wasps, silkworms, crickets, bamboo caterpillars, dragonflies and beetles are the most popular kinds of edible insects in many regions of China. However, most of the consumed edible insects are collected from the wild. People consider food coming from the wild as natural and healthy food. Another reason is the lack of appropriate technologies to farm edible insects. Therefore, edible insects are not a main source of food at present. Several factors have influenced the utilization of insects as food, such as consumer acceptance, food security and lack of large-scale farming technologies. We consider that the development of large-scale farming and process technology are the most important conditions for the advancement of an edible insect sector in China. However, several efforts have been made. Some wasp and locust species are farmed using semi-domestication methods. This is also true for mealworm and cockroach species. Currently the scale of rearing is too small to meet human food requirements at a large scale. Many efforts are still needed to reach a level of industrialization of edible insect farming

### Research and industrialization of Hermetia illucens L. in China

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Hermetia illucens L. (Diptera: Stratiomyidae), also known as black soldier fly (BSF), can be found in decaying organic wastes, such as animal manure and food waste. Over the past 14 years, investigators in China have conducted a tremendous amount of research on the BSF. In the beginning we imported the technique from Dr Jeffery Tomberlin, Texas A &M University, USA. Then, China collected wild species of H. illucens in Wuhan and Guangdong Province and domesticated them. Research found that the quartz-iodine lamp can replace natural light inducing BSF adults to mate and lay eggs in cloudy days. We also isolated microbes that could induce BSF to lay eggs, resulting in increased production. These innovations allow the mass production of black soldier fly larvae (BSFL) at a large scale throughout the year. BSF gut microbes play important roles in BSFL nutrition and resistance against pathogens. Bacteria isolated from the BSFL gut and eggs can be cultured and used to promote BSF growth, and shorten the time from hatching to the prepupal stage. BSF not only convert kitchen waste and livestock manure, but also could convert mushroom residues, soybean residues, silkworm faeces and algae waste. We found BSFL could also degrade antibiotics in livestock manure as well as remove odours in livestock manure. These processes greatly reduce environmental impact caused by organic waste. BSFL fat also could serve as a novel low cost biomass feedstock for biofuel. Industrialization of BSF for recycling kitchen and animal waste is well established in China. The treatment process have several models: manual, mechanization and automation model. Some companies in Wuhan city, Xian city, Tianjin city, Hainan Province, Hunan Province and Guangdong Province recycle kitchen waste and livestock manure at a large scale. Resulting products from the larvae generated include insect powder and live BSFL that can be used as animal feed ingredients. These ingredients have been used to feed catfish, frogs, and turtles at a large scale. The residues collected form BSFL are subjected to secondary fermentation using antimicrobial microbes to produce functional organic fertilizers and reach high-value utilization and zero emission of organic wastes. The future for industrialized use of BSFL for waste management and product development is very bright in China.

### Insects can not only 'feed the world'; they can also 'heal the world'!

Keynotes 4

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Insects, used as food, were first suggested to help ease global problems of future food security by Meyer-Rochow in 1975. However, insects and other invertebrates also play roles in traditional healing. Some uses may be based on the tenet of 'similia similibus', but not all non-conventional health promoting practices should be 'rubbished'. Practices that have stood the test of time ought to be investigated. One problem that doubters have regarding the validity of such treatments is that almost any therapeutically used species appears to have multiple uses. How could totally different organ systems benefit from extracts, potions, powders, secretions, ashes, etc. of a single species? Even though therapeutically used invertebrates are small, they possess organs for specific functions, e.g. digestion, gas exchange, reproduction and have a nervous system, endocrine glands, a heart and muscles and a multitude of different molecules like metabolites, enzymes, hormones, neurotransmitters, etc. exist in them. It seems likely that a single species prepared and used in different ways could have a multitude of uses. But how come different target organs apparently respond to a range of taxonomically not closely related species? How can asthma remedies involve earthworms, molluscs, termites, beetles, cockroaches, bugs, and dragonflies? Diseases are often based on the same essentials: viruses, bacteria, fungi, etc. and in cancers mutated, rapidly proliferating cell lines. Invertebrates themselves can suffer from infections and cancers and to fight a disease, unifying principles are likely to have evolved in all of them. It would seem far more surprising if each species had evolved its own unique disease fighting system. But we still need information from more regions and ethnic groups on medicinal uses of invertebrates like insects as western medicines become dominant and traditional healers are unable (and sometimes even unwilling) to transmit their knowledge to the young. Kunin and Lawton suggested that the highest priority for conservation be given to species involved in traditional remedies, but collecting and uncontrolled uses of insects can put undue pressure on certain highly sought after species. Mahawar and Jaroli therefore stressed that 'there is a need to shift the focus from how to obtain the greatest amount of zootherapeutical resources to how to ensure any future uses'.

## Edible insects and food security in Africa: needs and opportunities, environmental issues of edible insects

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Food and nutrition insecurities are among the major issues for the development of sub-Saharan African countries. The continent has the highest prevalence of undernourishment globally, with around one in four people undernourished. Protein-energy malnutrition is highly prevalent among young children resulting in diseases such as Kwashiokor and Marasmus. Furthermore, micronutrient deficiencies, particularly iron, zinc, iodine and vitamins, are alarmingly high among pregnant women and preschool children. Despite this precarious situation, considerable proportions of fish and sovbean produced and/or imported on the continent are directed towards animal feed production as protein sources. On the other hand, edible insects are rich sources of protein and some essential amino acids, energy and micronutrients of public health significance, and can therefore, play a key role in filling the food and nutrition gap, especially for the undernourished and marginalised communities. While it is estimated that more than 500 insect species are consumed in Africa by about 300 million people in 75% of the countries in Africa, entomophagy remains traditional, with seasonal wild harvesting, unsafe post-harvest handling processes and poorly established supply chains backed by little science-based interventions. Likewise, while 80% of chicken consumed in the continent is from traditional free-range insect fed stock, legislations are non-existent in the feed production sector to enable the use of insects in commercial animal feeds. Since 2013, the International Centre of Insect Physiology and Ecology (icipe) has led various upstream and downstream research and policy initiatives using insects as food and feed to promote food and nutrition security, improved health and job creation opportunities in a sustainable environment in Africa. This presentation will highlight the high potential implications of these initiatives.

## Promoting consumption of edible insects: a Kenyan perspective

Keynotes 6

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Kenya has a long and rich history of human consumption of insects in certain regions of the country; however, studies conducted recently report that consumers with no or little previous exposure to edible insects are quite resistant to the introduction of insects in their diet. In the past, most insects were collected from the wild habitats for domestic consumption, but insects are now increasingly being commercially produced and sold in rural and urban markets. Production patterns are evolving to include not only wild harvesting but also semi-domestication and insect farming due to efforts on improving small scale and large scale production systems. Players are employing a pull and push model where insect production has to be up-scaled and the demand for such products have to be increased simultaneously. Promotion activities have been supported by studies investigating the willingness of Kenyan consumers to consume insects, advocacy and sensitization efforts. Showcasing insects and insect-based products in trade fairs and exhibitions and talk shows on audio-visual media are complementing the efforts. Training of farmers to produce and eat insects have been going on by various actors in the country. Insects are being promoted not only as 'nutritious' but as 'good food' by the development of appealing and appetizing diets on the menu which minimizes phobia to the wider population. Start-ups producing nutritious and acceptable foods are emerging and the researchers are giving technical support. Some of the products are being marketed as protein-rich and targeting sections of the population with high protein demand. Production, processing, and marketing of edible insects is providing important income, employment and livelihood opportunities with new niche markets offering growing opportunities for entrepreneurs. While traditional household consumption remains important, consumption patterns are evolving, with increased demand for insects and insect-based products to the wider population.

## The contribution of high school students to succession and development of eating insect culture as a case of *Vespula* spp.

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This study reports the encouragement of the habit of insect eating, as a case of Vespula spp. in Japan by high school students. The program emphasizes youth and senior interaction. Such activities enable enthusiasm and promotion of insects as food. The data were collected though participant surveys of the Hebo club in Ena Agricultural high school located in Ena, Gifu prefecture, Japan as well as through semi-constructed interviews of students of the club and local people affiliated with the school since 2016. Historically, the habit of using Vespula. Spp. for food was common amongst people living in the mountainous region in Central Japan. Techniques of hunting these insects included following individual wasps to their underground nests and 'cultivating' the colonies until they are large enough to harvest. Various cooking methods have developed resulting in seasonal delicacies. In recent years, however, the number of individuals that practiced this behaviour has decreased. Locals are sensitive to the loss of their culture, which partly inspired the project being reviewed in this presentation. The Hebo club was established in order to promote such activities and sustain the culture. Hebo means Vespula spp., in the region where the Hebo club is located. The students have learned from the locals the techniques for finding, collecting, rearing, and cooking *Vespula*. Through these experiences, the students were able to collect information and organize it into an exhibit. Their vigorous activities have encouraged their parents and locals to learn more about their culture. Due to interest in this aspect of their culture, the students have made proposals for the further popularization through ecotourism or cultural study tours. These proposals have been recognized for their uniqueness as well as numerous data obtained from their studies and experience. The efforts by club members to protect the culture of their community has resulted in great pride by the senior generation in the region.

# A triangulated foundation to promote efficient and safe production of the black soldier *Keynotes 8* fly at an industrial scale

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The black soldier fly, *Hermetia illucens*, (Diptera: Stratiomyidae) has become established as one of the key insects being industrialized for use in waste management, protein and biofuel production. While many companies have demonstrated success with the mass production of this insect, key hurdles remain with regards to developing optimal efficiency, safety, and commercial strategy for supplying larger industries such as aquaculture. The objective of this presentation is to review these three criteria and propose potential solutions. For optimization, adult biology will be the primary focus as adults are needed to produce the larvae used in the system. However, once larvae are produced, ensuring they are safe for consumption will be critical along with producing sufficient quantities to meet the demands of a particular industry group. Polishing these facets of a key jewel of the insect industry will allow it to shine bright and serve as a model for other insect species being developed for similar uses.

### The future of insect proteins from a food system perspective

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Insect proteins have been mentioned as one of the most viable options in a report presented in Davos by McKinsey and the council on Food Security and Agriculture. The report addresses emerging and enabling technologies like robotics, advanced analytics and genomics and how they accelerate alternative proteins like insect proteins towards global availability. Low footprint and sustainable alternative proteins are needed in order to meet demand in the coming thirty years and innovations across regulatory frameworks, industry and society are all needed in order to achieve nutrient parity (affordable and good foods available for everyone). Insect proteins are becoming increasingly interesting to achieve such goals and actors in government, industry and NGO's are expressing and acting more concretely every day. What can be expected in the next years? What are differentiators from a feed and food perspective? How do insects play their part in the food system of the future?

## Insect industry development in Europe and beyond

Keynotes 10

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By 2050, the World Resources Institute projects a 70% human food calorie gap. The race for sustainable protein alternatives is heating up; livestock consume 20% of global proteins – in direct competition with human consumption for dwindling fish stock, water, land, and soil resources. To avoid global issues, the world needs massive productivity increases in protein production. The FAO expects insect protein could help close the gap. The European insect sector is an emerging industry which concentrates most research and innovation efforts that are invested into the sector worldwide. Legislative decisions taken by EU policy makers from 2015 onwards, as notably materialized by the revision of the EU legislation on novel food early 2018 or the relaxing of the EU feed ban rules authorizing fish feed market in 2017, constituted decisive factors which contributed to boost the advancement of the sector. The International Platform for Insects as Food and Feed (IPIFF) is an EU non-profit organization which represents the interests of the insect production sector towards EU policy makers, European stakeholders and citizens. IPIFF mission is to promote the wider use of insects as an alternative or new source of nutrients for human consumption and animal feed. Most EU producers use exclusively indoor systems, which allows for proper insect growth and development at large scale. They also developed advanced production techniques, based on the automation of most processes and the optimization of insect rearing conditions and diet formula. Among these players, Ynsect is a leading global provider of sustainable, premium nutrition for all by tapping the natural goodness of insects at large scale. Ÿnsect has designed proprietary vertical indoor automated farms that delivers first and foremost premium natural ingredients for aquaculture and pet nutrition, made of molitor beetles. This presentation will present the state of art of insect production in Europe and beyond, and will focus on major issues of insect industry development, i.e. technological, regulatory and commercial bottlenecks.

### Environmental impact of insects compared to other protein sources

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Insects use for food and feed should provide a more sustainable solution (than meat, plant-based or mycoprotein alternatives) to address protein deficit problems in many regions of the world. The selection of viable comparison units represents one of the challenges for reliable analyses. Environmental assessments and comparisons are not applicable for all the cases of insect production and use due to the early stages of insect production and processing technologies readiness levels (TRLs), diversity of species and nutritional composition. Quite a specific hurdle of insect-based food and feed life cycle assessment (LCA) lays in the absence of proved effective processing technologies. In most cases, TRLs are preliminary and represent a great variation of possible techniques for insects killing and slaughtering, chitin extraction or transformation, defatting and combination with other ingredients. The selection of processing methods plays a significant role for LCA score changes (up to 20-40%). Insect feeding diet is vital for LCA results as it could change the environmental impact in 28-60%. Higher impacts are especially evident for insects cultivated on high-protein feed grade material (4.8-5 kg  $CO_2eq.$ ; 2.4-3 m<sup>2</sup> of arable land; 40-51.2 MJ of non-renewable energy). Low feed quality leads to food security problems, which should be yet studied and understood. Feed challenges are associated with problems of land use changes accountability (responsible for the highest impacts for climate change in food production chains), information lack on generated wastes by production of insects and protein digestibility accounting. Despite multiple challenges, insect production is less environmentally impacting than meat (pork and beef) and mycoprotein meat analogues. Plant-based meat substitutes seems to be more beneficial at current state of TRL, however a holistic sustainability analysis considering all three dimensions (environmental, economic, social) need to be developed and applied. Indicated challenges of LCA demonstrate a great potential for production technologies improvement and further research of insect-based food and feed sustainability identification.

### The nutritional role of edible insects

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We – those of us who wish to – eat many different insects. Some are high in fat, while others are lean and packed with protein. To understand the nutritional impacts of insect consumption, we must rely on several sources, including: lab experiments detailing the breakdown of nutrients available in different insect species, dietary surveys that explore the contribution of edible insects in a wider dietary setting, and measurements of health outcomes in insect-eating and non-insect-eating groups. The first of these sources is by far the most widely available. In this paper, we review published data of all three kinds, and introduce the results of some recently collected field observations, interviews, focus groups and 24 hours dietary recall surveys of caterpillar consumption in West Africa. In light of all currently available data we conclude that insects can play multiple dietary roles, and that their nutritional contribution to our diets is dependent on how we choose to consume them. We end by highlighting the importance of more detailed studies to elucidate the observable health impacts of insect consumption.

## What if these tools could change your food habits: devices and methods to empower humans to co-create a healthy food system

#### K. Unger

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On a global scale, it is becoming increasingly hard to grow and consume food in a way that is sustainable for the planet and healthy for the people. Edible insects as ingredients can have a highly positive effect on the ecosystem and on people's health. But why should people engage in this movement? Livin Farms has developed tools and infrastructure for humans to grow, harvest and ultimately market insects as an ingredient for sustainable food applications. The first product is the Hive Home, a device that empowers people to become independent and grow their own protein and nutrients in the form of mealworms in their homes. It was shipped to around 350 customers world-wide and has since had an impact on these customers' lives, their eating habits, their perspective about insects as ingredients and their perspective on their lifestyle. Qualitative and quantitative data was gathered and the customer journey evaluated. Ultimately, the only ones who can have a positive impact on the food system are the people. People determine the way they grow food in the first place and people choose the final products in the supermarket. It is their desires and decisions that can make the food system healthier for the people and more sustainable for the planet. This is why Livin Farms is so concerned with all the human actors along the food chain. Beginning with the insect farmer on various scales cultivating the insects, over to the person who picks a product from the supermarket shelves and serves a cooked meal to his or her family, down to the person who decides to dispose the food remains. For such a novel field as insect ingredients, every part of the system, every interaction, needs to be carefully designed for people to engage successfully and integrate it into the food chain on a larger scale.

### Fad or trend – an honest outlook on the edible insect market in North America and beyond

Keynotes 14

### M. Ashour

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The hype around edible insects has grown to meteoric proportions in just the past four years. However, consumer adoption – though on the rise – remains slow. Why is this the case? In North America, over a dozen consumer packaged-goods (CPG) start-ups emerged in the past four years with the goal of creating mass-consumer adoption of insect protein. None have so far succeeded at a large scale. This talk offers a transparent and candid account of the state of affairs of the edible insect movement in the West in general and North America in particular. Drawing from the example of several similar emerging industries that succeeded in achieving mainstream adoption, the goal of this presentation is to provide a framework for leaders within the edible insect industry to approach mass-education and mass consumer adoption. This presentation will not focus on consumer behaviour, but instead on how business models must pivot and adapt to the reality of current consumer barriers.

### Women and edible insects: a deep, deep history

#### J. Lesnik

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Ethnographic accounts of hunter-gatherers eating insects suggest that women forage for insects more often than men and also receive a greater amount of nutrients such as protein derived from these resources. The difference in the utilization of this resource seems to fit the widespread pattern of differential resource procurement by the sexes in foraging populations whereby men tend to target higher-risk resources, such as hunted game, while women focus more on resources with a lower risk of acquisition failure. However, the evolution of the sexual division of labour is not fully understood. This pattern could have evolved because of benefits associated with having both parents provision for the family or it could have a more ancient origin related to the different sexes having different needs from the foods they target. To test which model fits best for edible insects, I test two predictions: (1) that insects offer nutrients that women need in greater quantities than men; and (2) that sex differences in insect consumption exist in other members of the order Primates, such as our close relatives the chimpanzees. A review of relevant literature demonstrates that although available nutrients are variable across insects, they often contain significant amounts of nutrients important to reproductive women, such as protein and iron. Additionally, there are multiple accounts from anthropoid primates (monkeys and apes) that supplement insects into their diets demonstrating that females consume insects more than males (notably chimpanzees, orangutans, and capuchins). Nonhuman primates do not pool their resources, so the increased consumption of insects by these females is likely related to a nutritional benefit. These findings suggest that insects provide a reliable nutritious resource for female primates and that the pattern seen for humans today likely has an origin much older than the nuclear family and paternal provisioning.

### Domestication of African gourmet caterpillars

Session 1 Ethno-entomology 2

### D. Ambühl

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Large saturniid caterpillars have been an important food source in Africa since time immemorial. However, wild insects have been lost in recent decades to overharvesting, habitat destruction, bushfires, and deforestation. This has posed serious problems for the supply of protein to the rural population, especially children and women, who traditionally collected these insects. For the first time in human history, attempts are being made in the Democratic Republic of the Congo to domesticate these caterpillars and to rear them as agricultural livestock. By means of practical breeding experiments and life cycle analyses with more than two dozen locally consumed caterpillar species, the most suitable for domestication will be evaluated. The project is being directed by the Congolese biologist, Augustin Konda, and run in a laboratory that was built in Kilueka in 2017. Initial potential candidates for large scale production have already been identified from the genera *Lobobunaea* and *Pseudobunaea* of the Saturniidae family. In the next three years low tech facilities and training modules for farmers will be developed. The principal objective is to improve food security and develop a sustainable and environmentally friendly production process for high quality food that is culturally welcomed. This could be a lighthouse project for other countries and communities in Africa because this method allows rich animal proteins, fats and micronutrients to be produced using a feed source that is not in competition with human consumption. This project is receiving significant support from the Salvation Army headquarters in London and Kinshasa.

## Review of past and current status of insects for food and feed in Kenya: reintroducing entomophagy

#### M.A. Ayieko

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Traditionally consumption of edible insects was a way of life in Kenya until western style of food preparation, food habits, and consumption patterns were introduced. African societies had not frowned at eating insects nor termed the practice dirty, primitive, or simply a poverty survival means, till the introduction of the western civilization in the turn of the 20<sup>th</sup> century. Following the gradual environmental changes that has attributed to land degradation and climate change, the challenges of food insecurity have triggered the research community to focus their studies on courses of such challenges and alternative sources of food. Research shows overwhelming empirical evidence supporting the use of insects as food and feed, their medicinal values and their contribution to environmental management. Studies show that Africans still consume considerable number of wild insect species and several insects are used for traditional medicinal purposes, during cultural festivities, while others are harvested to be used as feed for livestock or bait for small wild animals. Currently a lot of work is being done to ascertain consumption of the edible insects as healthy, safe and a secure way of providing animal protein for families in Kenya and many other countries. Novel method of processing, preparation and consumption are being researched to merge traditional and modern lifestyle. This review looks at the historical past usage of insects, current scientific work in the research fields, and the future prospective of edible insects in human and animal nutrition in Kenya. This is an attempt to identify the role of value addition of insects as food and feeds and sustainability of entomophagy.

### Research and development of Ophiocordyceps sinensis

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#### R. Han and L. Cao

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*Ophiocordyceps sinensis* (Ophiocordycipitaceae) is an entomopathogenic fungus endemic to the Tibetan Plateau. The fungus-insect complex is useful in healthcare but limited in the field, so urgently in need of artificial rearing. Large-scale artificial rearing of the *Thitarodes/Hepialus* insect hosts has been established in the low-altitude area. Both insect species, *Thitarodes armoricanus* and *T. jianchuanensis*, have long and unusual life cycles; it takes 263 to 494 days for *T. jianchuanensis* and 443 to 780 days for *T. armoricanus* to complete a developmental cycle, including egg, larval instars L1-L9, pupa and adult. The larvae can develop into pupae from the L7, L8 or L9 instar larvae. The insect larvae are infected by *O. sinensis* and mature fruiting bodies are harvested successfully. Successful artificial rearing of host insect species and the cultivation of this important fungus-insect complex at low altitudes will provide the effective protection of this bio-resource and supply commercial trade.

### Settlement behaviour of new queens of the weaver ant Oecophylla smaragdina

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The weaver ant (*Oecophylla smaragdina* Fabricius), a prominent and important economic edible insect of Thailand, is an eusocial insect species in the family Formicidae, order Hymenoptera. In this research, the effects of tree species, leaf characteristics, direction on the tree and height above the ground on the settlement site of new queens were studied. The data collection was conducted along the roadside in Pak Thong Chai and Wang Nam Khiaw district, Nakhon-Ratchasima province, Thailand, from May to June 2017. New queen observations were carried out by selecting the tree that located in an open area approximately 10-50 m from the known colony where the tree can get the sunlight from all directions. The trees with an open canopy structure with appearance of curled leaves or superimposed leaves and white silk present were selected. Four tree species were selected by the new queens. There are 37 individual new queens settled in 25 points on the canopy of five trees. However, some of them stayed in groups, maximum four. Two types of leaf characteristics were selected by the new queens: superimposed (64%) and curled (36%). The new queens were found in five directions of the canopy: west (56%), southwest (16%), south (12%), east (8%) and northwest (8%); all new queens were found on the apex of the branches. The height of 1-4 m (average 2.1 m) was the distance above the ground that the new queens and on weaver ant farming in the future.

## Industrial design of insect plants to achieve safe products with consistent quality Farming insects 1 Session 2 Farming insects 1

#### A. Baumann and F. Cordesmeyer

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When commercializing insect-based ingredients for food and feed, producers commit to a high level of responsibility regarding product safety. Food and feed safety incidents normally result in high financial losses, brand damage, and even closure of production sites for the implicated companies. When introducing new ingredients made from insects, consumers are likely to be even more sensitive to potential risks and a single incident may have severe consequences for the entire industry. Consequently it is crucial that every individual insect producer implements a preventive system with specific measures to mitigate biological, chemical, and physical safety hazards, thus avoiding any negative perception of insect-based products. In this presentation key food and feed safety concepts are outlined that need to be taken into account, when designing and building industrial plants to grow and process insects. Particularly the case of obtaining protein meal and lipids from organic residues with the help of black soldier fly larvae is addressed. When designing a new production facility, the first step is an adequately performed risk assessment. This includes the evaluation of all production inputs (e.g. feedstock, water, air), processing steps and outputs regarding potential hazards. A main source of potential contamination is the organic matter used to feed insects. In a case study using the hazard analysis critical control point (HACCP) concept, we identified the following critical control points to mitigate potential hazards within the production line: (1) pH-level during feedstock storage; (2) thermal decontamination of reared larvae; (3) moisture content of the protein meal; and (4) metal detection before product packaging. However, the foundation for a welldesigned HACCP concept is the implementation of good manufacturing practices (GMP), which includes sanitary building and equipment design, cleaning and disinfection procedures, and personnel hygiene. A particular focus in the presentation will be put on (1) hygienic zoning inside the building to prevent cross-contamination between raw and finished products; (2) cleaning and disinfection practices during larvae processing to reduce the risk of contamination; and (3) the importance of traceability through each stage of processing to guarantee trustworthy products. As a technology provider of industrial solutions for the farming and processing of insects, we hope that the presented guidelines will help to design large-scale insect plants resulting in high quality products that are safe to apply in food and feed.

### Utilization of Hermetia illucens larvae for the production of feed

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The larvae of the black soldier fly (*Hermetia illucens*) is a sustainable and resource efficient alternative protein as well as food and feed source. It can be utilized for the conversion and valorisation of a big variety of organic substrates and has a high potential for the application e.g. in the aquaculture as a fishmeal. Recent studies show its suitability in dog food, in terms of palatability and digestibility. The use in human nutrition is basically possible but not yet followed up in a high extend. Hermetia Baruth GmbH (HBG) started in 2006 with the production of black soldier fly and achieved the first stable and significant population in Europa. HBG is still the biggest producer in Germany of BSF larvae and the derived products like protein rich meal, fat and fertilizer on an industrial scale. An overview of the production process is given. It is divided into substrate acquisition and preparation, insect rearing and harvesting process. Besides the challenge to choose a cost-efficient as well as nutrient-efficient substrate, further optimization of the rearing conditions for the improvement of mating, oviposition and larval as well as pupal development is still researched. The core product is the protein rich insect meal, but also the by-products fat and fertilizer have a value and support the viability of the approach.

### A scalable black soldier fly production system

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The JM Green company and members of EVO Conversion Systems company have long been working together to improve the black soldier fly rearing system. The JM Green company investigates mechanical solutions in the automation process, while the EVO company is exploring the biological potential of black soldier fly larvae. With the combined effort of the two companies, many interesting things were discovered which will be shared.

## Development of nursery diets for the mass rearing of black soldier fly

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A study was conducted to determine an optimal nursery diet for the mass rearing of the black soldier fly (BSF) (Hermetia illucens). Various mass production facilities rear neonatal BSF larvae on chicken layer mash. The rearing of these animals for the first five days of their life cycle, also known as the nursery phase, was found to be suboptimal when reared on layer mash. Mortalities ranged between 20-60%. A nursery diet was formulated based on the amino acid composition of the neonatal larvae using the comparative slaughter technique. Newly formulated diets also tested blood meal as alternative raw material. The effect of a sterol source on the growth and development of neonatal BSF larvae was also evaluated. A total of 5,600 freshly hatched neonates were allotted to 56 pill pots. Seven treatments with eight replications were allocated to the pill pots in a randomized design. Treatments were: (1) diet formulated according to general nutrient specifications of laver 120 (control); (2) diet formulated according to general nutrient specifications of laver 120 including blood meal; (3) diet formulated to resemble amino acid composition of BSF larvae; (4) diet formulated to resemble amino acid composition of BSF larvae including blood meal; (5) diet one with the addition of a sterol source; (6) diet three with the addition of a sterol source; and (7) commercial layer 120 mash currently in use by factory. Diet 3 significantly increased survivability by 25% compared to Diet 1 and Diet 7. The inclusion of bloodmeal in the two treatment diets also caused a significant increase in survivability. The treatments (Diets 5 and 6) that included a sterol source yielded the lowest number of larvae but had a significantly higher average larval weight compared to all other treatments. A strong negative correlation was obtained between survivability and average larval weight. Certain key factors were observed in terms of physical properties of the diets that need to be addressed in future studies. Addressing these factors will ensure a more productive nursery diet for rearing BSF neonates.

### Can insects synthesize vitamin D after exposure to ultraviolet light?

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Worldwide over 2,000 insect species are used as a source of food or feed. In recent years systems to produce these insects have developed rapidly. Such systems usually employ artificial lighting conditions, which in most cases lack ultraviolet radiation (UV). It is well known that UV radiation can facilitate *de novo* synthesis of vitamin D in most vertebrate species. This vitamin then modulates calcium and phosphorus metabolism, regulates cell proliferation, differentiation and apoptosis, and plays a role in the innate immune system in these vertebrates. However, whether insects can synthesize vitamin D is currently unknown. Therefore, four insect species (yellow mealworms, house crickets, migratory locusts and black soldier fly larvae) were exposed to artificial UV. For each species half of the specimens were allocated to a UV exposure treatment (8 h/d; n=5) and the other half was housed under similar conditions, but without UV exposure (control groups; n=5). When the first insects in a container reached the (pre)-pupal or adult stage, samples were taken and analysed for their vitamin D content. Large differences in synthesizing capacity were found between the four species with house crickets attaining relatively low levels of vitamin D and yellow mealworms attaining high levels after UV exposure. Furthermore, also the form of vitamin D that was synthesized, i.e. vitamin D<sub>2</sub> or D<sub>3</sub>, differed between species. Whereas this study shows that certain insect species can synthesize vitamin D, further studies are required to determine which role this plays in their physiology.

## Potentials and barriers for insect farming in Kenya

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The overall aim of the GREEiNSECT research project was to investigate the potentials and barriers for the development of insect farming in Kenya, to contribute to food security, improved nutrition and a 'greener' food system. The research addressed the key knowledge gaps for sustainable production of crickets and black soldier fly (BSF) in Kenya: technological aspects of optimizing production systems (species, local feed sources, diseases, etc.); barriers for adoption in rural communities; consumer perception and acceptability of insect-based foods; impact of insects on nutrition and health; BSF for waste treatment and fish feed; and the environmental impact of cricket relative to chicken (LCA). The results showed that the current production systems introduced in Kenya have promising potentials but needs to be optimized, especially for efficient utilization of local feed sources; most consumers in Kenya are unfamiliar with insect-based food products but are interested and willing to adopt; the environmental burden of insect farming is low. A multi-sectoral approach across the value chain is needed for successful scaling-up of the insect sector in Kenya. The GREEiNSECT project was a collaborative research partnership between University of Copenhagen, Denmark and universities in Kenya (Jaramogi Oginga Odinga University of Science and Technology (JOOUST) and Jomo Kenyatta University of Agriculture & Technology (JKUAT)) as well as international partners such as International Centre for Insect Physiology and Ecology (Icipe). More information and publications available on www.greeinsect.ku.dk.

## Cricket farming in Asia by Cricket Lab

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#### N. Bery

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For the past few years, Nicolas has tried to perfect the process of making cricket flour from crickets with his previous company. He has worked with local farmers to source his crickets and develop a supply chain and transform the insects in his facility. Inevitably, he came to face different problems with supply quantities and consistency. Thai cricket farms are not well equipped to provide enough crickets for the growing market with a consistent quality and moreover the price is not adapted for mainstream adoption. The conclusion was to build a new project to tackle these issues. This is when Cricket Lab was born: a pilot cricket farm with its own processing facility, that will be the base to develop and improve farming and processing techniques for future large scale operations. The project has overcome many problems already and is past its own first production. The future development of the company aims to continue innovation through their R&D centre in Thailand and prepare for large scaling of production. This part will be done directly with local communities in Thailand and South East Asia by transferring our technology and knowledge acquired through our researches. The presentation is about the hurdles of starting a large-scale cricket farm and future obstacles that the project will most probably face but also the impact we expect to have on local farmers.

## Technical efficiency of a cricket farm in Kenya

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The study investigated the economic efficiency of use of inputs and how they affected productivity within a cricket farm established at Jaramogi Oginga Odinga University of Science and Technology (JOOUST), Kenya. Using Cobb-Douglas stochastic frontier analysis, it further evaluated the sources of inefficiency in production. Feed and labour were found to positively influence efficiency of the production, while change of species from *Gryllus bimaculatus* to *Acheta domesticus* increased inefficiency. The farm was undergoing decreasing returns to scale when production was scaled up and thus, the study concluded that the technology used in production needed to be adjusted for productivity to improve.

## Effect of rearing substrates on the fitness parameters of newly recorded edible *Farming insects 9* cricket *Scapsipedus marginatus* in Kenya

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Recently, efforts to mass produce native cricket species as a viable option for a more sustainable food source to enhance food insecurity has gained momentum worldwide. The paucity of scientific data and the reluctance of breeders to share detailed information have considerably hampered cricket farming in Africa. Our study reports the first record of the edible cricket Scapsipedus marginatus (Afzelius & Brannius, 1804) (Orthoptera: Gryllidae) in Kenya and investigate the effect of six food substrates on their development, survival and reproduction. The developmental duration and survivorship of the nymphs differed significantly across the different food substrates. The shortest developmental duration (66 days) and highest survival rate (93%) was recorded when S. marginatus was fed wheat bran meal, while the longest developmental duration (103 days) and lowest survival rate (46%) was recorded on maize meal. Cricket reared on maize and carrot meal had a significantly higher pre-oviposition duration (11 and 10 days, respectively) compared to those reared on the other diets with <8 days. The highest fecundity per female was observed when crickets were fed on soya bean meal  $(1,402\pm25 \text{ eggs})$ , followed by wheat bran  $(1,248\pm37 \text{ eggs})$ . The percent hatchability was significantly higher on soya bean meal (94.8±0.8%) and wheat bran (92.0±1.0%). The highest adult longevity (52.4±2.0 and 50.2±1.8 days for male and female, respectively), wet weight  $(0.68\pm0.02 \text{ g for males and } 0.9\pm0.03 \text{ g for females})$  and body size (2.2±0.03 g for males and 2.5±0.10 g for females) was recorded on wheat bran meal. Female-biased sex ratio was recorded on wheat bran, soya bean and carrot meal, whereas it was male-biased on maize meal. Our findings revealed that S. marginatus is a potential candidate for mass rearing when suitable diets are provided. Thus, further research on nutritional ecology of combining different food substrates into high-quality nutrient diet composition is crucial to efficiently produce crickets that meet the nutritional requirements for humans or other animal.

### Insects for resilience of family farming: perspectives, challenges, and scenarios centred around biomass transformation

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Traditional agriculture has usually been based on the multicrop-livestock farming model. It is still the main model worldwide and usually the prerogative of family-centred peasantry. Optimizing resources is a key aspect of these practices, as land, labour and capital are often limited. This leads to a need for fine tuning farm management and their close environment. Biomass plays a central role fulfilling key services such as energy, soil fertility or livestock feed. Modern agricultural trends tend to favour mechanization, heavy fertilizer and pesticide use and overspecialization as well as the separation of production elements. Coupled to modern agriculture, these give rise to increasing agricultural wastes which are hard to deal with, and paradoxically to the underutilization of surrounding biomass: 998 million tonnes of agricultural wastes are produced every year. Insects are great biomass converters with a wide range of possible feed, even when focusing only on species suitable for food and feed. Only a few are being bred and even less so on a large scale. Farming insects presents multiple advantages including high feed to protein ratio, low water consumption, and fast lifecycles which make them an important part of agricultural waste management systems and sustainable biomass management systems. The aim of this paper is to investigate a set of scenarios drawing from pioneer experiments and companies already undertaking research and projects with Hermetia illucens, Rhynchophorus ferrugineus, Encosternum delegorguei, Cetonia aurata, Ergates faber, Ruspolia differens, Cirina forda, Gryllodes sigilatus and Macrotermes bellicosus, focusing on converting readily available biomass. Both developed and emerging economies will be considered: France, Benin, Burkina Faso, Laos and Cambodia. From there we advise several possibilities for amelioration, among which the need for a better database of species, new species domestication and new farming techniques to include insects in human agroecological systems.

#### Session 2 Edible insect farming: a strategy for providing sustainable nutrition and economic Farming insects 11 empowerment for orphanages in the DRC

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The Democratic Republic of Congo (DRC) is one of the poorest and most food insecure countries of the world and the country's five million orphaned children face particularly grave chronic hunger and malnutrition. Realizing a need for a sustainable and economical solution to the persistent challenge of under-nutrition in orphanages, Farm for Orphans and Global Orphan Foundation launched an insect farming initiative in 2017. We reviewed literature on insect nutritional composition and insect farming technologies to develop an integrated strategy to provide orphanages in DRC a lowcost, technologically feasible alternative for protein production. Larvae of the African palm weevil (*Rhynchophorus phoenicis*) were targeted as the primary 'micro-livestock' crop due to their favourable nutrient profile, low resource utilization, and traditional role in the Congolese diet. We developed a farming protocol that was implemented within four orphanages; the orphanages were trained in rearing techniques, supplied with farming materials and provided technical support. Laboratory analyses were conducted to compare the nutritional composition of the farmed larvae with wild-harvested larvae available in Congolese markets. Insect farming was found to be technically and economically feasible in orphanage settings. Early data suggest that a small-scale orphanage farm can produce up to 2,800 g of palm weevil larvae every ~40 days. The nutritional profile of the farmed larvae is comparable to wild-harvested larvae. Thus, farming palm weevil larvae within orphanages can provide a year-round increase in the availability of calories, protein and critical micronutrients to children in their care. Further, we found that a small insect farm in Kinshasa can generate ~USD \$300/month in income. Edible insects provide critical nutrition to many of the world's poor, however they are typically wild-harvested and not widely farmed; further, limited data exist on effective insect rearing technologies or the benefits of insect farming in food-insecure settings. Preliminary results of our initiative are promising, suggesting that palm weevil farming allows capital-poor orphanages to grow their own sustainable and nutritious food while offering the potential to generate supplemental income through the sale of surplus yields. Future initiatives include aiding our orphanage partners in expanding their farms, staggering cycles such that a weekly larvae harvest is achieved and improving access to local markets. Our strategy may be used as a model for other populations in resource-poor settings where entomophagy is practiced.

## Insect farming: a socially sustainable component of the agricultural sector?

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From sustainable consumption and production to reduced inequality, the agricultural sector plays a significant role in the achievement of the Sustainable Development Goals (SDGs). Approximately 1.1 billion people are involved in this sector, making agriculture an excellent starting point if we are to achieve the SDGs globally, regionally and nationally. New ways of thinking and doing are in high demand. Though consumed and used throughout history in many regions of the world, insects for human consumption and animal feed are now receiving an inconceivable amount of attention worldwide. Insects are more efficient at converting food into edible mass because of physiological and biological differences. This means that they emit lower amounts of greenhouse gases when compared to traditional livestock species. At present, seven life cycle assessments have evaluated the environmental sustainability of these production systems. However, regarding the other pillars of sustainability, no study has systematically evaluated the social sustainability of insect farming. A 2017 study in Thailand indicated that cricket farming provided farmers with an additional livelihood that was perceived as economically rewarding, easier to carry out than other agricultural tasks and suitable for a variety of ages and capabilities. Another study in Kenya found that 71% of cricket farmers are women and that insect farming can be a source of economic empowerment. At regional and national levels, many governments support the innovation and growth of insect farming systems and are working to accommodate this development with suitable policies. Recognising the social impacts of food production systems is equally as important as the environmental impacts; however, measuring them is a challenge. One way to do this is through social life cycle assessment, a novel methodology that is used to evaluate the social sustainability of processes. However, the assessment of the social impacts of production processes is still at a development stage due to fragmentation and a lack of empirical foundation. A critical reason for this is the absence of general standardized indicators that reflect and measure the social impact of a business along product life cycles and supply chains. Building on this methodology is therefore critical to improving the social sustainability of the agricultural sector. This presentation investigates and discusses potential impact categories that could be used to evaluate the social impacts of insect farming around the world. Social impact assessments will most like gain greater significance as the insects for food and feed industries expand in scale in the future.

## Developing a service for diagnosing and managing insect diseases to assist the growing insect industry Session 3

#### J. Eilenberg and A.B. Jensen

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The rapid increase in insect production for food and feed in Europe and elsewhere in the world has led to a need for a coordinated action to assist producers in the diagnosis and management of insect diseases in production stock. Diseases caused by viruses, bacteria, fungi, protozoa and other insect pathogens can be detrimental for reared insects and may cause significant economic loss to producers. Academia, commercial companies and other insect producers should jointly develop best practice for diagnosing insect diseases early and thereby manage such diseases efficiently. We present plans for a European coordinated action, the challenges to address, and how different European laboratories specialized in insect pathology should collaborate. An important issue will be to educate a new generation of insect pathologists, who with a combination of classical insect pathology methods and the most modern tools can become professionals in diagnosing and providing guidance how to manage insect production systems to minimize the risks of infections by insect pathogens.

#### Microsporidian pathogens of mass-reared insects – a future threat to edible insect cultivation? Session 3 Insect diseases 2

#### S. Bjornson

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Whether insects are reared as food for human consumption or as animal feed, insect mass-rearing systems often encourage overcrowding, temporary starvation and cannibalism. Such stressful conditions often enhance the prevalence of insect pathogens. Microsporidia are common, spore-forming pathogens that cause chronic disease. The earliest record of a microsporidian pathogen was reported from silkworm, and since then, microsporidia have been reported from many other insects, including some insects that are often consumed as food by humans. Microsporidia often debilitate their hosts, and there are numerous reports of these pathogens causing reduced longevity and egg production, developmental delays and abnormalities, and increased mortality. Because infected individuals often remain asymptomatic, microsporidia frequently remain undetected in insect mass-rearings until there is a noticeable decline in production. There are effective means of screening and monitoring insect cultures for microsporidia, and some effective control measures. These rely on early detection before these pathogens become widespread in a rearing facility.

#### Opportunistic bacteria as pathogens of mealworms

Session 3 Insect diseases 3

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There are multiple production conditions that are important for insect large-scale rearing systems. These conditions include population density, temperature, relative humidity and diet quality, among others. When the equilibrium among some of the production conditions is broken, the possibility for opportunistic pathogens to trigger epizootics increases exponentially. Opportunistic bacteria, for example, have been described as pathogens of different insect species reared under laboratory conditions. Mealworms, which are produced in large numbers under closed rearing systems and utilized as a source of protein and other food and feed ingredients, particularly in Europe, are susceptible to opportunistic bacteria. Opportunistic bacteria of special interest are *Serratia* spp. and *Pseudomonas* spp. that can also become human pathogens. A real case of epizootics caused by an opportunistic bacterium in a commercial rearing was reported in January 2015 when a strain of *Pseudomonas aeruginosa* was detected in a giant mealworm (*Zophobas morio*) colony. The characteristic symptoms of the disease included feeding cessation, melanisation, flaccidity and dead. Detection and taxonomic identification was done by biochemical and molecular analysis. A report was delivered to the company with the findings of the diagnosis and recommendations on how to increase the chances for controlling the bacterium in the system.

# A bacterial pathogen in cricket farming: identification, biology, and suggestions on Insect diseases 4 Insect diseases 4

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One important challenge in insect farming is to diagnose and manage insect pathogens. Pathogens present in rearing systems often hinder optimal productivity due to reduced biological quality of the infected insects and the expected lower product yield. In severe cases, insect pathogens may cause the rearing to collapse. Identifying such pathogens, investigating their possible ways of entry into production facilities and determining the factors that trigger their proliferation in these facilities are thus essential. Such information will allow us to establish measures to control insect diseases or even better, to avoid pathogens. A bacterium infecting crickets was found in 2017 during a survey of cricket pathogens in a number of insect farms in Kenya and Uganda. This bacterium infects different species of crickets. The characteristic symptoms of diseased individuals include highly swollen abdomen, viscous yellow opaque hemolymph and partly dissolved body tissues. Morphology of the bacterium resembles members of the genus Ricketsiella when examined with the transmission electron microscope. Molecular analysis has been initiated for more specific taxonomic identification.

## Life cycle assessment of insect production systems: lessons learned and the way forward

Session 4 Environmental impact 1

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Life cycle assessment (LCA) is an important tool to understand the environmental impacts of production systems, including those of livestock. Despite insect farming is recognized and promoted as a sustainable alternative to conventional livestock, to date, fewer than ten LCAs have been conducted on insect production systems, and mainly on pilot scale or experimental production systems. This presentation looks at the importance of LCAs in shaping our understanding of the contribution of insect production to sustainable food production systems and diets. Lessons learned from a LCA study of full-scale commercial cricket farming compared to industrial-scale broiler production in Thailand, conducted under the GREEiNSECT project, will be discussed and a way forward for future studies will be presented.

## Greenhouse gas emissions from fly larvae composting with Hermetia illucens

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Rapid increase in waste generation and slow implementation of adequate waste management is a global phenomenon. Large proportion of municipal solid waste (MSW) generated globally is either not collected or disposed of by open dumping and landfilling. Considering that over 50% of the MSW is organic matter (MOW), the landfills and dumps are the major contributors to the global anthropogenic greenhouse gas (GHG) emissions, responsible for up to 45 Mt of the global  $CH_4$  emissions to the atmosphere. Monitoring of GHG emissions from waste treatment options is thus key to selecting the alternatives that have the smallest negative environmental impacts. Current widespread treatment alternatives for MOW can either result in large GHG emissions, loss of nutrients or lack generation of valuable products. Fly larvae composting (FLC) is a waste treatment method, which allows biomass recycling and generation of high-value products. While FLC is able to generate valuable products and preserve nutrients, the knowledge on its GHG emissions is limited. FLC treatment relies on the ability of larvae to convert the biomass in the waste to their own biomass. Typical food waste partly consists of substances which are slow to digest. Pre-treating of food waste with microorganisms able to break down these substances has potential to improve the degradability of food waste and increase the biomass conversion efficiency. The aim of this study was to measure the emissions of GHG such as CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O during FLC food waste treatment at a laboratory scale and to evaluate the effects of waste pre-treatment on the biomass conversion efficiency and the GHG emissions. The FLC was performed in small containers with 700 larvae in each, resulting in larval density of 2 larvae/cm<sup>2</sup>. The gases were sampled using a closed chamber four times within one hour and were analysed using gas chromatography. The gas flows were calculated based on the gas concentration increase within the volume of the chamber over time. The tree different FLC treatments resulted in very small GHG emissions ranging from 0.01 to 0.04 kg CO<sub>2</sub>-eq./tonne treated material (WW), and from 0.02 to 0.14 kg  $CO_2$ -eq./tonne treated material (WW) of  $CH_4$  and  $N_2O_2$  respectively. The  $CO_2$ emission ranged from 52 to 59 kg CO<sub>2</sub>-eq./tonne treated material (WW) within the different treatments. The influence of pre-treatment was insignificant, but nonetheless resulted in a greater overall material reduction. The results suggest that FLC as a food waste treatment alternative has potential to significantly lower GHG emissions comparing to other waste treatment alternatives, while also generating valuable products in form of larval biomass and treatment residue.

## Black soldier fly larvae tolerating and degrading naphthalene, fluorene, phenanthrene and pyrene

Session 4 Environmental impact 3

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Polycyclic aromatic hydrocarbons, widely distributing in environment, have detrimental biological effects, toxicity, mutagenicity, and carcinogenicity, to ecosystem and human. The black soldier fly, *Hermetia illucens* L. can consume a wide range of organic pesticides and has the potential to be used in improving environment. In this study, four kinds of typical polycyclic aromatic hydrocarbons were spiked into the larval feed, then their degradation and the tolerance of black soldier fly larvae were evaluated. The results indicated these chemicals resulted the increase of larval developing time and decrease of larval weight and eclosion rate significantly. However, the larvae were still alive in those contaminated substrates and their mortality rates were not significantly different with those of control. After larval treatment, the average degradation rate of naphthalene, fluorene, phenanthrene and pyrene was 40.4, 23.3, 22.7 and 14.6%, respectively, approximately 1.7 times higher than that of control. The larvae reared in the artificial feed had the commendable ability to consume polycyclic aromatic hydrocarbons. It suggests the potential use of black soldier flies as an environmental cleaner.

## The degradation of tetracycline by black soldier fly (Diptera: Stratiomyidae) Session 4 Iarvae with intestinal microorganisms

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Tetracycline (TC) widely exists in livestock manure and may present challenges for black soldier fly larval (BSFL) degradation. The ability and mechanism of BSFL with associated intestinal microbes to degrade TC was investigated in this study. Results indicate Non-sterile BSFL digested TC mass 96.9±0.4% for initial concentrations of 40 mg/kg. However, sterile BSFL were less efficient with TC degradation recorded at 38.2±7.3%. We hypothesize intestinal microbes of BSFL could accelerate TC degradation rate. Four tetracycline antibiotic resistance genes, *tetG, tetM, tetO* and *tetX,* were detected in the intestine of BSFL from the TC treatment; data indicate three primary TC resistance mechanisms may work together for BSFL intestine microbes to resist and degrade TC. Six BSFL intestinal microorganisms, BSFL-1 (*Serratia* sp.), BSFL-2 (*Trichosporon* asahii), BSFL-3 (*Pichia kudriavzevii*), BSFL-4 (*Candida rugosa*), BSFL-5 (*Galactomyces geotrichum*) and BSFL-6 (*Serratia marcescens*) were isolated and tested for TC degradation *in vitro*. All six targeted bacteria degraded TC efficiently in LB medium by 28-50% in over four days. These data demonstrate microorganism associated with BSFL digestive tract could play a crucial role in reducing pollutants associated with larval resources.

## Performance of pre-weaning kids fed a milk replacer containing either cricket or black soldier fly

Session 5 Food production systems 1

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This research was designed to evaluate the effect of milk replacer containing cricket and black soldier fly (BSF) flour on performance, blood haematology and metabolites of pre-weaning kids. The experiment used a completely randomized design with sixteen kids and four treatments. The treatments were goat milk (GM), cow milk (CM), milk replacer containing cricket meal (CMR) and milk replacer containing black soldier fly larvae (BMR). Variables observed were nutrient intake, blood profile, blood metabolites, physiological status and performance. Result showed that in all treatments there were no significance differences in physiological status (respiration rates, heart rate and body temperature) and metabolite parameters (glucose, triglyceride and total protein). Nutrient intake of CMR was higher (P<0.05) compared to the other treatments. Haemoglobin, haematocrit, and red blood cells of GM and CMR treatments were higher than CM (P<0.05), but white blood cells and differentiation of leukocytes were not affected by the treatments. Meanwhile, the performance of CMR was higher than BMR. In conclusion, milk replacer containing cricket and BSF flour have good palatablity and can replace goat milk to support of the growth rate of pre-weaning kids.

### Product development of food with edible insects

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The work focusses 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 snacks or minimeal 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 inVALUABLE project – to implement the flavours 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: (1) functionality testing of defatted insect protein – emulsifier, foaming agent, water and lipid binding; (2) investigation of fat extraction techniques; (3) analysis of fat composition; and (4) application testing – bread, beverages, spreads, and snacks. As part of this, the taste of different edible insects (primarily mealworms) have been evaluated with the aim of aiding the right application for each insect type in food. To develop the right products, both consumer testing through questionnaires and sensory test as well as market research has been performed. The major results will be included in the presentation.

### Could we produce even more healthy insects for humans?

Session 5 Food production systems 3

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Edible insects are considered as sustainable and sometimes healthier alternative for other animal protein sources. Insects do contain large amounts of minerals, vitamins, fatty and amino acids essential for humans. Enhancing the nutritive value of livestock products with feeds is a known way to increase the beneficial factors. This could be achieved with insects as well but in more drastic way with smaller input. Low saturated and higher polyunsaturated fatty acid content is beneficial for humans. To improve this aspect of the nutritive value of edible insects, there is a need for understanding to what extent the fatty acid composition and content of insects can be modified. Some insects are able to store dietary fatty acids largely unaltered to their fat body and this suggest the possibility of modifying their nutritional composition by using artificial diets. We tested how artificial feeds with manipulated content of essential fatty acids influence the fatty acid composition and content of *Ruspolia differens*. This East African edible grasshopper is very nutritious containing even up to 60% fat of dry weight. We reared individuals in laboratory with seven artificial feeds where essential fatty acid, saturated fatty acid, carbohydrate or protein levels had been maximized. According to our findings, it is possible to drastically modify the fatty acid composition and content of *R. differens* with diet. Our results also show that omega-3 fatty acid content can be increased to 45% of total fatty acid content with diet. Even up to 50% of R. differens' weight gain occurs during first ten days after adult moult. This suggests that very short-term feed boost after adult moult might be effective to achieve changes in polyunsaturated fatty acid content. Our findings can be used to sustainably improve human nutrition by producing edible insects with designed nutritional content.

# Eating insects: a sustainable livelihood assessment of insect consumption Food production systems 4 and harvesting in Kenya, Kakamega

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Edible insects are highly nutritious and can increase food security as well as generate cash income; therefore they are an important and often freely available natural resource for rural households in Africa. This study advances our understanding of human consumption and harvesting of insects as a strategy to reduce undernourishment and foster local agricultural growth in sub-Saharan Africa. Based on household surveys, in-depth interviews and participatory data collection in two rural villages in Kakamega County, Western Kenya, we investigated the benefits and challenges of insect consumption, using sustainable livelihood theory as the analytical framework. Our study shows that 96% of the 98 surveyed households rely on harvesting termites for nutritional and economic reasons, but to different extent. They collect on average 675.5 kg during the two termite seasons and consume around 17% while selling the rest. The average income generated from selling termites per household per season is \$21; making it possible for households to cover part of their basic needs for oil, soap, and blankets. Unfortunately, the households experience decreasing availability of insects, blaming changing rain patterns and chemical applications to nearby sugar plantations run by large agri-enterprises. Regional research efforts into insect rearing, rather than wild harvesting, may provide a resourcesolution through small-scale productions of insects. With high prices of insects recorded on urban markets, a steady production of insects would provide not only a source of nutritious food, but also generate a needed cash flow. As shown in other studies of consumption of forest/wild products, insect consumption may also have an important role as a safety net and gap filling for rural households. Small-scale insect production may further be a path out of poverty, and being a more sustainable protein source compared to other meat products, insect rearing and consumption needs more attention and research.

#### Development of an inVALUABLE insect sector in Denmark

Session 5 Food production systems 5

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Since the 1<sup>st</sup> International conference on Insects to Feed the World in May 2014 in the Netherlands, the insect sector in Denmark has undergone intense development with the involvement of private and public stakeholders throughout the value chain – from production to products. Over the last couple of years, this development has accelerated even further, driven to a large extend by the national innovation project inVALUABLE (Insect Value Chain in a Circular Bioeconomy). inVALUABLE is one of the largest publicly-funded R&D projects in Europe on insects as feed and food. The project involves 10 partners and runs from January 2017 to December 2019 with a total budget of 3.7 M EUR. The vision of inVALUABLE is to create a sustainable resource-efficient industry for animal protein production based on mealworms. The partners span the entire value chain and include entrepreneurs, experts in biology (entomology and nutrition), biotech, automation, processing and food tech and -safety. Overall, inVALUABLE addresses three major challenges for the insect industry: (1) upscaling of production to industrial level, (2) regulatory issues and (3) consumer acceptance. The presentation will showcase the most recent results of inVALUABLE. This will include e.g.: (1) optimization of production of *Tenebrio molitor* (e.g. diet and other rearing conditions); (2) evaluation of protein quality in mealworms across different processing methods using the protein digestibility-corrected amino acid score (PDCAAS) based on rat trials; (3) regulatory progress with the national authorities and subsequent amendments to implementing production of food grade insects; (4) food innovation and application; and (5) communication efforts to support 'cultural change management' amongst the Danes.

### Innovative insect production - food from wood

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A process for composting woody plant waste to produce edible insects is currently under development in Switzerland. Cellulose is the most abundant, naturally-produced material on planet Earth and is in fact chemically stored solar energy. Unfortunately, this high-energy, natural substance cannot be used directly as food for many animals and humans. Fungi, however, use an enzyme to split cellulose into carbohydrates that can be metabolised. Additionally, many wood-dwelling insects carry microorganisms in their intestinal tracts which can use the high-energy cellulose and, under certain circumstances, fix atmospheric nitrogen. Over the next four years, the Zurich University of Applied Sciences will work on developing a process for cultivating Cetoniid and Dynastid larvae using specially prepared substrates of fermented wood and plant waste as part of a project called 'Food from Wood'. The project is funded by the Swiss Federal Office for Agriculture and aims to build and test a pilot facility for producing insects using wood and wood-containing plant waste.

## Honey bee drone brood for human consumption: creating awareness for a discarded resource

Session 5 Food production systems 7

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Current evidence indicates that the practice of drone brood removal is an effective measure of *Varroa* spp. control in honey bee colonies when combined with other treatment as part of an integrated pest management (IPM) strategy. This has led to a widespread adoption of the method in Denmark and other European countries. Drone brood is considered as nutritious and delicious, but currently mostly discharged by the beekeepers. To examine the potential use of drone brood as a food source on a commercial scale, we gathered data from nine Danish apiaries. The weight of drone brood comb removed from each colony was recorded and from one apiary, the edible biomass was determined. The total weight of the drone brood comb removed from each colony over the season was highly variable ranging from 0.184 kg to 4.035 kg with an average of 1.776 kg and the average total drone brood biomass extracted was 1.064 kg per colony. We conclude that, with a potential 80 tonnes of available biomass nationally, drone brood could be used as a food product within a specialised niche market and foster sustainable beekeeping.

## Glucosamine hydrochloride from silkworm (*Bombyx mori* L.) pupae shell inhibit *in vitro* human plasma lipid peroxidation

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Glucosamine has been widely used to treat osteoarthritis (OA) and suggested as an antioxidant in the OA treatment. The aim of the study was to analyse the antioxidant activity of glucosamine hydrochloride from silkworm (*Bombyx mori* L.) pupae shell on the inhibition of human plasma lipid peroxide *in vitro*. Lipid peroxidation was measured by the level of thiobarbituric acid reactive species (TBARS). The samples of plasma from 10 apparently healthy subjects (5 females, 5 males) were incubated with hydrogen peroxide (2 mM) and glucosamine hydrochloride (0, 10, 20 and 40 mM). Glucosamine hydrochloride significantly inhibited the  $H_2O_2$ -induced TBARS generation in a concentration-dependent manner (*P*<0.05). The level of TBARS was reduced by higher the concentration of glucosamine hydrochloride. The addition of glucosamine hydrochloride (10, 20 and 40 mM) in plasma significantly reduced levels of TBARS at 33.23, 49.65 and 58.51%, respectively, as compared with control (*P*<0.05). This results indicated that glucosamine hydrochloride from silkworm pupae shell has the ability as an antioxidant to inhibits *in vitro* human plasma lipid peroxidation.

## The bioconversion capability of *Hermetia illucens* larvae: a morphofunctional study of the larval midgut

Session 6 Feed production systems 1

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The black soldier fly (BSF), Hermetia illucens, is among the most promising insect species for the reduction of organic waste. Moreover, the high nutritional value of larvae and pupae makes them an interesting and sustainable protein source for the production of animal feed. Despite the great interest toward this insect species, the current literature mainly provides information on the rearing methods of BSF and indications on its use for waste treatment, while little is known about *H. illucens* biology. In particular, a deep understanding of the physiology of the midgut, which is implicated in food digestion and nutrient absorption, is essential to better comprehend the extraordinary dietary plasticity of the larva, which is able to grow on different food substrates. The main aim of the present study is to provide a detailed structural and functional characterization of the midgut of last instar larvae by using morphological, biochemical, and molecular approaches. Our results demonstrate that the larval midgut is composed of three distinct anatomical regions with different luminal pH. The midgut epithelium is formed by different cell types that accomplish nutrient digestion and absorption, acidification of the midgut lumen, endocrine regulation, and growth of the epithelium. The activity of the enzymes that are involved in protein and sugar digestion (i.e. trypsins, chymotrypsins, and amylases) is associated to specific districts of this organ. According to the evidence collected, we propose a functional model of the larval midgut of *H. illucens* in which each region is characterized by peculiar features to accomplish specific functions. This work represents the first morphofunctional characterization of the midgut of *H. illucens* larvae and sets the stage for the best exploitation of the bioconversion ability of this insect. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

## An analysis of the nutritive value of the black soldier fly larvae reared on different substrates

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An analysis of the nutritive value of the black soldier fly larvae (BSFL) reared on different substrates. Demand for meat is expected to double by 2050. This poses a major challenge to the world's ability to produce enough protein rich livestock feed. Insects have been identified as potential alternatives to the conventional protein sources due to their rich nutrients content and can be reared on organic side streams. Substrates derived from organic by-products are very suitable for industrial large-scale production of insect meal. Thus, comparison of the nutritive value of BSFL reared on four different substrates was conducted. The four substrates included; chicken manure (CM), brewers' spent grain (SG), kitchen waste (KW) and cow dung (CD). Samples of every substrate were collected for chemical analysis before and after the feeding process. Metallic trays measuring 23×15 cm were filled with 1 kg of each of the substrates. Fivehundred (500) neonatal BSFL were placed carefully in the trays on the respective substrates, which moisture content was maintained at 60-70% by carefully sprinkling distilled water, and reared for a period of 3-4 weeks at 28±2 °C and  $65\pm5\%$  relative humidity. Substrates were replenished with fresh weekly. Once the insects reached the prepupal stage, the larvae were harvested, using a 5 mm mesh size sieve. A sample of 200 grams was weighed from each replication and pooled for every substrate and then frozen at -20 °C ready for chemical analysis. Samples of the larvae and the substrates were analysed for dry matter (DM), crude protein (CP), ether extracts (EE), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), amino acids, fatty acids, vitamins, flavonoids, minerals and aflatoxins. The data was then subjected to analysis of variance (ANOVA) using general linear model (GLM) procedure. Bon-Tukey's procedure was applied to separate the means at P<0.05 level of significance. The results indicate variance in the nutrient composition as a result of rearing the BSFL on difference substrates. Crude protein, EE, minerals, amino acids, ADF and NDF were all affected by using different rearing substrates but not the levels of vitamins. BSFL fed on different substrates exhibited different accumulation patterns of minerals, with chicken manure giving the largest turnover of minerals. Though low concentrations of heavy metals (cadmium and lead) were detected in the BSFL, no traces of aflatoxins were. In conclusion, the nutrient load from the larvae reared in the kitchen waste was relatively higher compared with the others.

## Optimal feeding strategy of the BSF larvae for biomass production and organic waste reduction in Kenya

Session 6 Feed production systems 3

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Urban centres of developing countries are characterized by poverty, informal settlements, and non-collection of organic wastes due to lack of economic incentives for concerned stakeholders. The black soldier fly (BSF) valorisation technology transforms organic waste into a nutritious biomass is a financially attractive alternative method for sustainable disposal of organic wastes. This study aimed to establish a feeding strategy for production of Kenyan strain of BSF on faecal sludge supplemented with local organic substrates. Larvae feeding experiment was used to investigate effects of feeding rates, regimes and substrate combination. Data for larval growth, bioconversion efficiency and prepupa nutrient content were collected. Biomass yield, substrate reduction, and maturation days at feed rates of 100, 150, 200 and 250 mg/l/d were 124.0 $\pm$ 3.0 g, 81 $\pm$ 8.9%, 16; 140.0 $\pm$ 1.3, 84 $\pm$ 0.3%, 16; 176 $\pm$ 3.3, 57 $\pm$ 1.1%, 17 and 190 $\pm$ 1.2 g, 54 $\pm$ 1.2%, 20, respectively. Daily feeding regime produced higher biomass yields, substrate reduction and shorter maturation period (201 $\pm$ 7.4 g, 84.6 $\pm$ 0.2%; 16 days) compared to lump-sum feeding (204 $\pm$ 2.1 g; 77.1 $\pm$ 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 150-200 mg/l/d as the optimal strategy for waste management and biomass production.

#### Technical and biological aspects in the BSF mass rearing: how to reduce Feed production systems 4 variation?

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Predictability and diminishing variation are key factors in the commercial mass rearing of BS. Several examples will be given how we control our input streams and count and dose the larvae in the crates. Climate control in rearing cells is very important for a predictable output. Examples of cycles will be given, with emphasis on temperature, ammonia and CO<sub>2</sub> levels. The fat content of larvae during development and the relation between starvation and fat content will be discussed. Finally some aspects of the larvae and compost harvesting will be presented.

#### Ventilation requirements in a modular black soldier fly larvae system for waste treatment and feed production

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Session 6

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A great portion of the organic waste produced globally is not treated properly, mainly due to costs. As a consequence, the organic waste is often landfilled or dumped as it is the cheapest alternative in the short-term; but, this causes problems in the long-term with air and water pollution. Fly larvae composting (FLC), using larvae of black solder fly (BSF, Hermetia illucens), allows for the conversion of the organic waste fraction into high value protein and oil, while at the same time supplying a quick and efficient waste treatment alternative. Establishment of FLC in Sweden is performed under special circumstances; while there is an established treatment system available in Sweden for segregated organic waste, its products often have low value. Moreover, the current organic waste management system is not flexible enough to handle seasonal waste streams or to easily adjust to shifting waste volumes. Both of these flexibilities can be offered by a modular waste treatment system. Currently, a FLC modular treatment system is being developed at the Swedish University of Agricultural Sciences. The modular system comprises many smaller sub-units that each can treat 15 kg food waste. The sub-units are stacked and placed into a standard shipping container, yielding a treatment capacity of 500-1000 kg of organic waste per day. Upon completed treatment, the larvae are separated from the treatment residues. For an efficient separation, the residue moisture content should be low. The aim of this study was to investigate the aeration requirements for a modular FLC waste treatment system in an enclosed environment to simulate the conditions in the container. Residue moisture content of ≤50% was aimed for, in order to facilitate the separation. The evaluation was performed based on 15 kg treatment sub-units. Three stacks of 9 sub-units (40×60 cm crates) were placed in a closed cabinet with controlled ventilation. The temperature and humidity were monitored for incoming, outgoing and internal air in the cabinet. Material temperature was monitored with sensors at different heights in the cabinet. Based on sensor data and material samples the moisture removal rates and aeration rates were estimated. The minimal aeration required was found to be 8  $m^3/h$  per sub-unit to achieve a 50% water content of the treatment residue when treating food waste with a water content of 85%. This is equivalent to a requirement of removing 11 kg of water per 15 kg of treated material. The energy requirements for treating food waste with FLC technology can thus be greatly reduced if the initial water of the substrate is reduced.

### The design and application of automation equipment for pilot-scale black soldier fly composting of swine manure

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Annually China produces approximately 0.58 billion tonnes of swine manure. The great amount of manure wastes generated globally from the swine industry has caused serious environmental issues, especially diffuse pollution and the emission of greenhouse gases. Traditional composting is a common method for handling swine manure. However, the major disadvantage of this process is that nearly half of the carbon and nitrogen sources in manure wastes are lost as gaseous substances. The black soldier fly (Hermetia illucens L.), which can be used as feed, is able to use solid organic wastes as substrate. Bioconversion from swine manure into animal feed and fertilizer via black soldier fly larvae is a promising approach for manure recycling. There are rare reports about the design and application of automation equipment for black soldier fly composting swine manure. We designed an automation equipment for pilot-scale black soldier fly composting of swine manure. This equipment consists of a mechanical part and a control system. One unit of the mechanical part includes four layers fixed in a 2.5 m high frame. The upper three layers are made of three grooves sized  $10 \times 1.8 \times 0.35$  m (length, width, height). The under layer is a conveyer belt for collecting and delivering maggots for composting. The three units consist of one entire production line sharing one manure delivery system and a composting collection system. There is one m interval between two adjacent units for discharging, but the conveyer belt is continuously delivering the maggots on the vibrating screen. The other key mechanical part are stirrer motors installed at the upper layer of the whole production line. Each of these motors has a scraper (1.8 m long and 0.35 m wide), which is horizontal when stirring and vertical when discharging. This stirrer motor move along two racks placed on the sides of the groove horizontally when stirring or discharging. The whole control system includes five parts: manure allocation one time and inoculation of the naive larvae (0.3-0.5 g/100 larvae); stirring one time and manure allocation three times; manure allocation every six hours, if the temperature is higher than 42 °C and stirring; stirring every two hours, continued to one day; discharging on screen. With one production line (three units) of this automation equipment, about 3.24 tonnes raw swine manure (78% moisture) was decomposed thoroughly per day. One hundred fifty kg black soldier fly larvae was obtained from one tonne of piglet manure, and 120 kg larvae from 1 tonne of hog manure, and 110 kg larvae from one tonne of sow manure.

## Fed-batch cultivation of *Hermetia illucens* L. on almond by-products: impacts of feeding rate and inoculation density

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The by-products of almond production represent one of the largest agricultural residue streams in California. Work in our lab has demonstrated that black soldier fly (*Hermetia illucens* L.) larvae can be cultivated on almond hulls and shells (hulls) when moisture content and aeration rate are controlled. The competition for hull substrate by microorganisms that co-colonize hulls with larvae also needs to be considered in the provision of nutrients and process management. Experiments were performed to examine the role of larvae inoculation density and fed-batch processing on larvae growth. Inoculation density was varied from 1.5 to 6.2 mg larvae dry weight/g dry weight hulls (23 to 99 larvae/10 g dry weight) on day 0 and the remaining 96% on days 1,2,3 and 6 of cultivation ( $4 \times$  treatment). The other group of reactors was fed 3% of the total substrate on day 0 and the remaining 97% on days 2 and 6 of cultivation ( $2 \times$  treatment). As inoculation density from 3.1 to 9.9 mg/g had little effect on average larvae weight at harvest. At larvae inoculation densities between 3.1 to 9.9 mg/g, the  $4 \times$  fed batch treatments yielded greater larvae harvest weights than the  $2 \times$  fed batch treatments. However, at inoculation densities less than 3.1 mg/g, the  $2 \times$  fed batch production can improve larvae growth, but the rate of feeding needs to be selected in the context of larvae inoculation density.

## Cultivation of *Hermetia illucens* L. on almond by-products: impacts of moisture, nitrogen, and particle size

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Global human population is rapidly increasing, leading to a higher demand for food production and waste management. Innovative approaches to utilize crop by-products is critical to a sustainable future. Almond production is growing worldwide as well as its by-product, hulls, and shells. There is an opportunity to use this almond by-product for insect production. Studies were performed to determine if black soldier fly larvae (*Hermetia illucens* L.) could be produced on almond hulls and shells while examining the impact of moisture, carbon to nitrogen ratio, and particle size on larvae growth. Increasing moisture between 45 and 62 wt% wet basis increased average growth by 22% and increasing moisture above 60% increased average yield by 76%. An optimum carbon to nitrogen ratio for larvae growth was found to be 29 using urea as a nitrogen source. Larvae growth was consistently higher on course hulls and shells compared to fine hulls and shells. While black soldier fly larvae could be produced at a wide range of moisture contents, carbon to nitrogen ratios and particle sizes, these factors should be optimized for production. These results demonstrate that almond hulls and shells can serve as a suitable feedstock for black soldier fly larvae production.

## Temperature-dependent development, survival and reproduction of black soldier fly *Hermetia illucens* (Diptera:Stratiomydae)

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Efforts to recycle organic side streams using black soldier fly (BSF), Hermetia illucens (L.) into high-nutrient biomass that constitutes a sustainable high-quality protein ingredient in livestock feed have recently gained momentum worldwide. However, there is little knowledge of the most suitable environmental conditions for growth and development, which is a prerequisite for developing mass rearing technologies. We evaluated the temperature requirements for growth and reproduction of *H. illucens* on two diets [spent grains supplemented with brewers' yeast (D1) and un-supplemented (D2)]. The development rates at nine constant temperatures (10-42 °C) were fitted to a nonlinear temperature-dependent model and to a linear day-degree model. Life-table parameters were determined at optimal temperature for population growth within a range of favourable temperatures. Nonlinear estimates of thermal maximum (TM) for the larval, pre-pupal and pupal development ranged between 37.2±0.3 and 44.0±2.3 °C. Estimation of TM based on pre-pupal development was slightly lower at 37.2±0.3 °C on D1 and 38.0±1.0 °C on D2. Linear and nonlinear estimates of the lower temperature development threshold for the larval stage were 12.3±1.4 and 11.7±0.9 °C for D1, and 11.7±3.0 and 10.4±1.7 °C for D2, respectively. The thermal constant for total development of the immature stages of *H. illucens* was significantly higher for the larval stage (250±25 DD for D1 and 333±51 for D2) than the other stages evaluated. The final larval wet weight was significantly higher on D1 compared to D2. The most favourable temperature for population growth was 30 °C with significantly higher intrinsic rate of natural increase ( $r_m$ =0.127 for D1 and 0.122 for D2) and shorter doubling time (5.5 days for D1 and 5.7 days for D2) than at the other temperatures. These results are valuable for the optimization of commercial mass rearing procedures of BSF under various environmental conditions and for constructing computer simulation models to predict their population dynamics.

## Preliminary result of high fibre material impact on growth performance of black soldier fly larvae (*Hermetia illucens*)

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Black soldier fly (BSF) is a generalist species, its larvae can survive on different kind of decaying organic matter. This research wanted to investigate the impact of high fibre diet (stover corn: SC) on the development of BSF larvae and prepupae. Corn production is estimated around the world in 1,041 billion metric tonnes and only in China around 224.9 million metric tonnes. The availability of SC could be potentially relevant. A standard crop diet (Gainesville diet) was used as control (C). Three diets were formulated with a substitution of 25% (SC25), 50% (SC50) and 100% (SC100) of control diet with SC. Four replicates per treatment were performed and the replicates were fed daily with 40 g on a wet basis (70% moisture) in a climatic chamber with 28 °C T and 70% RH. Preliminary results showed that SC100 negatively impacted larval growth performance (a weight of 0.053 g after 42 days of trial) and were therefore excluded from the statistical analysis. Final length of larvae did not show differences between treatments and ranged between 16.4 mm (SC50) and 18.2 mm (C). Final weight of larvae showed differences between C and SC50 treatment: 0.128 g (SC50) and 0.170 g (C), respectively. Final prepupae weight of SC50 showed differences with C and SC25: 0.089 g (SC50) and 0.131 g (C), respectively. Time to reach prepupae of SC50 (20.8 days) was statistically different from C and SC25 (18.2 and 17.5 days, respectively). A diet completely composed by SC seems not suitable for the rearing of BSF larvae while a 25% of inclusion could be used. From an economic point of view, SC could be interesting in the view of a circular economy. Nevertheless, further investigations are needed to fully evaluate the effects of this low value substrate on larvae composition.

## *De novo* transcriptome sequencing and analysis revealed molecular basis of rapid fat accumulation by black soldier fly

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Black solder fly (BSF, Hermetia illucens L.), a high fat containing insect, has attracted wide attention in recent years. Although we have a lot of research on using BSF fat for biodiesel production, until now, the molecular mechanisms regulating fat accumulation by BSF are still unclear. It is essential that transcriptomic analysis identified the vital regulators involved in fat accumulation, and reveal the molecular basis of rapid fat accumulation by BSF for development of insectival biodiesel. We measured and analysed the dynamic patterns of fat content and fatty acids (FAs) composition of BSF larvae in eight different developing stages. We measured and analysed the fat content and fatty acids (FAs) composition across these life stages and examined the *de novo* transcriptome as wekk. By Illumina sequencing analysis, 218,295,450,000 nt bases are generated totally. In the results of assembly, 70,475 unigenes were detected, average length of unigenes is 1,064 nt, N50 is 1,749 nt. Meanwhile, a total of 9,159 up-regulated and 10,101 down-regulated differential expression unigenes were identified by DESeq method. The specific unigenes encoding known enzymes involved in fat accumulation in developing BSF were identified, and the temporal transcript patterns were analysed. In addition, four key metabolic genes involved in BSF fat accumulation were experimentally validated by quantitative real-time PCR (qRT-PCR). Our study systemic clarified the molecular regulatory mechanism of the rapid fat accumulation in developing BSF. This is the first study of transcriptome sequencing and analysis for fat accumulation mechanism in developing BSF. Meanwhile, this study may also provide some reference for researching the insectival biodiesel and laid a foundation to use the genetic engineering approaches to increase the fat accumulation and alter the fatty acid composition of insects in the future.

## Effect of bacterial supplementation on black soldier fly growth and conversion

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Black soldier flies (Hermetia illucens (L.) (Diptera: Stratiomyidae), BSF) are non-pest, non-biting flies that are not known to vector disease agents. Larvae occur in organic environments ranging from decaying food scraps, to trash bins, and even decomposing bodies. Aside from uses in composting and waste management, BSF larvae have been used as feed in aquaculture. With relative low production cost, easy rearing, and non-mechanized harvesting, dried larvae are a prime candidate to supplement existing feeding routines for both fish and poultry. However, nutritional requirements for optimal production of protein and oil from BSF have not been refined, nor is it known whether probiotic amendment will aid to enhance BSF digestion, nutrition, and conversion. We have addressed this question by supplementing a grain diet with two different bacterial strains, on which black soldier fly larvae were reared. We measured BSF growth and fatty acid compositions, as compared to the no-supplement control. Result showed BSF larvae fed with probiotic led to a significant increase in larval weight and faster development compared to control. Larvae fed with bacterial supplementation also metabolized more of the diet than when fed diet alone. Furthermore, analysis of fatty acids revealed that bacterial supplementation increased short chain fatty acids, while reducing the amount of unsaturated fatty acids, and showed detectable levels of vaccenic and eicosatrienoic acids, which are unsaturated fatty acids implicated in health benefits. Altogether, these data showed that bacterial supplementation of food has the potential to increase BSF larval lipid and protein content, feeding efficiency, and waste conversion. Results of this work provide understanding of the microbial contribution to BSF waste conversion and nutrition, and will provide a foundation for manipulation strategies to enhance larval feeding rates, and protein and oil production, highly impacting food security, environmental quality, waste management, and sustainable energy.

## How does starving of black soldier fly (Diptera: Stratiomyidae) larvae impact its bacteria?

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Symbiont bacteria must maintain a balance with its host in terms of nutrient production and utilization. The linkage between these bacteria and host stage is poorly understood, especially with regards to insect species used for recycling waste to produce protein. Deciphering potential shifts in metabolic activity associated with host bacterial communities under harsh conditions, such as starvation, could potentially lead to discoveries of mechanisms regulating the sensation of hunger. Once known, manipulation of the host feeding behaviour could be achieved and result in more efficient protein production and associated waste recycling. Black soldier fly larvae (BSFL) are the crown jewel of the insect-farming industry for the production of protein. Thus, this species is given a lot of attention for its potential of transforming organic waste into valuable protein within a short period of time. Associated bacteria communities of BSFL are known to be impacted by the food substrate provided to the larvae; however, the impact of larval starvation on these communities is not known. In this study, we induced starvation in BSFL and identified a dramatic bacterial community shift. With this discovery, we are now positioned to determine the mechanistic responses of these bacteria to starvation stress.

## Impact of diet nutrition and moisture on the bacterial community associated with *Hermetia illucens* (L.)

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Past research has revealed the intimate relationship between black soldier flies (*Hermetia illucens* (L.) (Diptera: Stratiomyidae) and bacteria. In this study, we investigated how diet nutrition, moisture, and black soldier fly feeding impacted the structure and function of the bacterial community associated with the diet. Microbial structure was assed using Illumina<sup>®</sup> MiSeq and function (Microbial Metabolic Community Profile) was assessed using Biolog EcoPlate<sup>™</sup>. Shannon diversity and bacterial community structure (genus level) were significantly affected by diet protein-carbohydrate ratio and moisture. Many of the bacteria associated with high protein diets were facultative anaerobes. Function was significantly affected by protein-carbohydrate ratio, moisture, and feeding activity of black soldier flies. This study brings a new component, nutrition ecology, to the industrialization of black soldier flies for waste management and protein production, and indicates this is an important factor to consider as it impacts not only the insects, but also the structure and function of the associated bacterial community.

## Primary studies on the diversity of culturable bacteria in the intestinal tract of the larvae of black soldier fly

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Black soldier fly (BSF), Hermetia illucens is a species with widespread distribution throughout the world. BSF larvae can feed on different organic wastes. They also are able to tolerate various contaminants in their food substrate. This species is a good candidate for converting organic waste into valuable biomass that can be used as animal feed. The present study evaluated the diversity of culturable bacteria in the intestinal tract of BSF larvae. Eight BSF larvae (two weeks after incubation) were surface sterilized with 70% ethanol followed by rinsing in sterile distilled water twice. Intestines of these larvae were dissected, homogenized, and placed on ice. The diluted homogenates  $(10^{6}-10^{8})$  were inoculated into MM4 medium under aerobic condition and YCFA medium under anaerobic condition and then incubated at 30 °C, respectively. In total 80 strains were isolated from both aerobic condition and anaerobic condition. These bacteria were affiliated with 15 genera such as Klebsiella, Enterococcus, Providencia, Alcaligenes, Citrobacter, Pseudomonas, Bacillus, Sphingobacterium, Morganella, Ochrobactrum, Acinetobacter, Paenalcaligenes, Miniimonas, Paenochrobactrum, Cronobacter and a genus in Verrucomicrobia. Among these isolates, Klebsiella, Enterococcus, *Citrobacter* are bacteria found in the intestines of many insect species, indicating they may play important roles in the metabolism of their host. Interestingly, a bacterial strain isolated from both aerobic and anaerobic conditions shared sequence similarity less than 94% in its 16S rRNA gene with other strains in the genus Miniimonas, which is possibly a new bacterial genus in the family *Beutenbergiaceae* of the phylum *Actinobacteria*. The physiological characteristics and functions of this bacterial strain and the other bacterial strains in the metabolism and development of black soldier fly are worth to be further studied. This study was supported by NSFC31670004.

# Life-history traits of the housefly, *Musca domestica* L. (Diptera: Muscidae), *Feed production systems 16* reared on three manure types

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Larvae of the housefly, *Musca domestica* L. (Diptera: Muscidae), have the potential to remedy two major concerns – managing animal wastes and feeding the world – as they are able to convert manure into valuable biomass. However, to date, houseflies have received limited attention as waste management agents in the United States despite the fact that large volumes of manure are produced annually. Therefore, this study aimed to examine the life-history performance of houseflies fed three different manure types as a possible solution for waste conversion. Treatments consisted of 100 1-day old larvae fed either 9 or 18 g of swine, dairy, or poultry manure every other day until 40% pupation. Gainesville diet was used as a control and produced superior results across all parameters examined. When comparing across feed rate for a manure type, larvae, in general, weighed more (4-15%) when provided more resource. A similar trend occurred for pupal weight (15-22%), adult weight (7-24%), and adult longevity (7-24%). Irrespective of feed rate (9 or 18 g), results demonstrate that larvae reared on dairy manure produced the heaviest final larval weight (0.0174 and 0.0191 g, respectively); yet, those fed swine manure reached the pupal stage faster (1-2 and 0.3-0.5 days, respectively) and resulted in the highest percent pupation (58 and 66%, respectively). Still, those fed poultry manure resulted in the heaviest pupae (0.0136 and 0.0158 g, respectively), adult male weight (0.0097 and 0.0119, respectively), adult male longevity (1.7 and 2.0 days, respectively), adult female weight (0.0102 and 0.0128 g, respectively), and adult female longevity (1.8 and 2.3 days, respectively). Data from this study is necessary to understand the development of houseflies fed manure in order to advance industrial systems for managing animal wastes.

## The promising roles of microbiota in the black soldier fly (Hermetia illucens) mass rearing and industrial applications

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The potential of black soldier fly (*Hermetia illucens*, BSF) as an efficient agent for organic waste treatment and novel protein production has been widely recognized globally. In the process of eliminating pollution, we could obtain high protein and high-fat insect biomass, which can be used as high quality animal protein feed and new lipid material to produce biodiesel. But limited understanding of its biology and behavioural restrict the mass rearing technology development and waste management application. The purpose of this presentation is to describe the roles of microbiota in BSF behaviour of oviposition, egg development and hatching, and larval development. We also examined the microbial diversity associated with each stage of development.

## Black soldier fly larvae (*Hermetia illucens*) bridges waste to resource: a full-scale operation

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Enormous amounts of food waste and animal manure are produced globally. Developing cost-effective and valueadded management of these wastes has received considerable public, academic, and political attention throughout the world. Vermicomposting using black soldier fly (BSF) larvae (Hermetia illucens) have been gradually highlighted as a promising biotechnology for these wastes management, generating bio-fertilizer, and larval protein. In 2016, collaborations with ZheJiang University, Shanghai Institutes of Biological Sciences (CAS), and HuaZhong Agricultural University, HangZhou GuSheng Bio-tech company set up a full-scale greenhouse-assisted BSF vermicomposting farm with daily treatment capacity of 15 tonnes of wastes. This BSF farm is being operated by a innovated flow process: waste pre-treatment and fermentation, continuous feeding technique for larvae bioconversion, larvae-residues separation, secondary composting of vermicompost, and BSF breeding. Currently, this BSF biotechnology provides an alternative animal feedstuff (i.e. BSF larvae, 41.5 and 38.5% protein and total fat as dry matter, respectively), as well as captured nutrients for agricultural re-utilization. Reductions in total weight, moisture, and total nitrogen in solids are found over 75.0, 80.6 and 70.5, respectively. In Oct 2017, we collected samples of raw food waste, larval gut, vermicompost in this full-scale BSF farm by using time-course protocol, and analysed with 16S rRNA sequencing, SourceTracker, PICRUSt and network analysis to decipher the metabolic function of larvae gut microbiome for waste biodegradation. The changes of metabolic functions and associated genes were significantly correlated with the succession of the associated microbial community. Moreover, bacteria that proliferated in vermicompost after larvae included Corynebacterium, Enterococcus, Vagococcus, Anaerococcus and Providencia. A systematic and complex interactions occurs between the larval gut and vermicompost bacteria over time through invasion, and differentiated into a new intermediate niche in favour of waste biodegradation. Based on theory of recycling economy, GuSheng company set up an extension and outreach centre with demonstrations of vermicompost-applied vegetable production, larvae-fed aquaculture and poultry farm, and popularization of science. It is concluded that BSF technology with subsequent production of value-added bio-products is beneficial for deep development of sustainable waste-to-resource recycling economy.

#### Inclusion of *Hermetia illucens* larvae to the diet of broiler quails: effect on immunity and caecal microbial populations

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A trial has been conducted to determine the effects of black soldier fly (BSF) larvae meal reared on two different substrates (100% commercial layer chicken mash (BSF-M) or 50% commercial layer chicken mash + 50% fish offal (BSF-F)) on certain immune parameters and on selected bacterial counts in the ceca of broiler quails. For this purpose, a total of 60 birds (20 birds/treatment) for immunological purposes and 30 birds (10 birds/treatment) for caecal bacteria counts were randomly allocated to one of three treatments diets: a control diet (CON) or a larvae meal diet containing either 10% BSF-M or BSF-F larvae meal. Fish offal were chosen to form part of the substrate for larvae in order to increase the content of long chain omega-3 (n-3) polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both known for their immunomodulatory properties. To determine the humoral immune response, quails were injected twice with porcine red blood cells (PRBC) and serum antibody titres were determined to represent the primary and secondary humoral immune response. As a measure of cell-mediated immunity, thickness of the wing web was measured 24 hours after injection of phytohemagglutinin-P (PHA-P). Lysozyme concentrations, bactericidal activity and protein fractions of the serum were determined after receiving dietary treatments for 27 and 37 days. Quails in the BSF-M group had a significantly higher secondary humoral immune response compared to the CON group, with BSF-F being intermediate. Dietary inclusion of larvae meal significantly increased cell-mediated immunity, with the BSF-F group exhibiting the greatest response. Dietary treatments had no effect on serum bactericidal activity. The majority of serum protein fractions were not influenced by treatment, with the exception of  $\alpha^2$ -globulin and  $\gamma$ -globulin. For both sampling dates,  $\alpha^2$ -globulin was significantly higher in the BSF-M and BSF-F groups compared to the CON group. The γ-globulin levels for quails in the BSF-F treatment group were significantly lower than the BSF-M and CON treatment group for the first sampling date, but levels increased after 10 days, resulting in concentrations being comparable between treatments. Lastly, feeding the dietary treatments for 20 days had no significant effects on the caecal bacterial counts. To conclude, dietary inclusion of larvae meal has immunostimulatory effects in broiler quails, but the substrate used to rear the maggots on, most definitely plays a role in the outcome.

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## Use of house flies as poultry feed by smallholder farmers in West Africa: prospects and constraints

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\${protocol}://www.wageningenacademic.com/doi/pdf/10.3920/JIFF2018.S1 - Wednesday, May 30, 2018 1:34:27 AM - Universita' Statale di Milano IP Address:159.149.162.67

In West Africa, traditional poultry production suffers from a lack of protein feed, resulting in high chick's mortality, slow growth and low fecundity. A majority of smallholder farmers give termites to their poultry, but the quantities available are usually too low to fulfil the protein needs. Surveys in Benin, Ghana and Burkina Faso showed that five to nine percent of poultry farmers feed their poultry at least occasionally with house fly, Musca domestica L. (Diptera: Muscidae). Various organic wastes are exposed as substrates to attract naturally occurring flies, and large quantities of larvae are produced in just three or four days. The substrates most commonly used by farmers are chicken and pig manure, maize and soy bran, and rumen content. in the IFWA project, over 80 different substrates and mixtures of substrates were tested for their suitability to produce larvae. Also the addition of attractants (e.g. blood, fish offal, etc.) was tested, as well as the importance of the container shape and material. At the same time, data were collected on the local availability and costs of the substrates. This study showed that many substrates, of both vegetal and animal origin, are potentially suitable but their cost and availability are highly variable among regions and even among villages within the same region. Thus, palettes of potential substrates should be proposed to villagers, who could then choose those that are locally most appropriate. Before being promoted, the fly production methods need to demonstrate their safety for the producers, the animals and the consumers. In particular, studies are presently being carried out to ensure that fly production does not increase the prevalence of human and animal diseases carried by the adult flies. Alternatives using black soldier flies, Hermetia illucens (L.) (Diptera: Stratiomyidae), and house flies kept in confinement are considered, but their profitability and implementation in villages are challenging.

## Dietary Madagascar cockroach meal on growth performance of broiler quails

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The objective was to evaluate the effects of dietary inclusion levels of Madagascar cockroach Gromphadorhina portentosa meal on the growth performance of broiler quails Coturnix coturnix coturnix. The study was carried out at the quail rearing facilities of the Federal University of Minas Gerais (UFMG), Montes Claros/MG, Brazil. Madagascar cockroach meal was obtained from a commercial producer (Vidaproteina LTDA, Nerópolis, GO, Brazil). A total of 312 quails of both sex were distributed in a completely randomized design, with 4 dietary treatments (0; 6; 12; e 18% inclusion level of insect meal) and 6 replicates. All diets were formulated to meet the minimum requirements for quails. Feeds and water were provided *ad libitum*. Growth performance parameters were evaluated in two periods, from birth to 21 days old and from birth to 35 days old. Results were subjected to ANOVA and when significant, regression analysis was performed using SAS 9.4. Differences were considered significant when P<0.05. During the initial growth (birth to 21 old days), treatments did not significantly influence feed conversion ratio (FCR). However, final weight, weight gain and feed intake were significantly influenced by diets, according to equations  $y = 152.11 + 2.71x - 0.078x^2$  (R<sup>2</sup>=0.82);  $y = 143.52 + 2.49x - 0.067x^2$  (R<sup>2</sup>=0.83); and y = 13.36 + 0.15x (R<sup>2</sup>=0.67), respectively. The optimal inclusion level of Madagascar cockroach meal in the diet of broiler quail for maximum weight at 21 days old is 17.40%. For maximum weight gain, from 1 to 21 days old, is recommended 17.80% inclusion level of Madagascar cockroach meal. From quails birth to 35 days old, the final weight and weight gain were not influenced by treatments. However, feed intake and FCR increased as the level of dietary Madagascar cockroach meal increased, according to equations y = 2.73 +0.02x (R<sup>2</sup>=0.34) and y = 2.73 + 0.12x (R<sup>2</sup>=0.46), respectively. The Madagascar cockroach seems to be a potential feed ingredient especially during early growth stage of quail. The authors would like to thank Vidaproteina for providing the Madagascar cockroach meal and Brazilian research funding agencies FAPEMIG and CNPq for financial support.

# Replacement of soy using insect meal in a commercial free range egg Chilean company

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Black soldier fly is one of the most efficient insect species in transforming organic waste into sustainable feed ingredients and specifically protein. Sheppard *et al.* evaluated the use of BSF protein for different type of animal species, including poultry. The objective here is to study the effect of diet formulation on eggs, incorporating insect meal with a 25, 37 and 50% replacement of soy, and measuring different parameters such as egg posture, egg size, hen weight amongst others. The specific objectives are: (1) to identify the macronutrients, fatty acids and amino acids in a BSF meal sample; (2) to formulate four different balanced and isoenergetic diets, with three different replacements of soy using insect meal; (3) to gather consumption indicators, posture index, conversion index and mortality of all diets; and (4) measure the functional (fatty acids) components transfer to the eggs. The trials are for a commercial free range company that is evaluating to incorporate an 'insect fed' brand to its products.

## Does insect-based feed lead to higher profit in layer chicken enterprises?

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Insects form part of the natural feed of traditional chicken across the world. In Africa, the indigenous free-range chickens contribute to over 80-85% poultry meat and 20% of eggs consumed. However, across the continent, insects are not approved as feed ingredient in commercial feed production. The present study aimed at evaluating the potential of black soldier fly larvae (BSFL) as a novel alternative protein ingredient in layer chicken feed to inform policy and revise standards in Kenya and Uganda. Black soldier fly larvae inclusion levels of 0, 50, 100, 150 and 200 g/kg (corresponding to 0, 25, 50, 75 and 100% fish and soybean meals substitution levels, respectively) were evaluated over a period of 22 weeks using 31 weeks-old KARI improved indigenous layer chicken. Parameter measured included feed intake, egg weight, egg production, egg yolk colour and body weight change. Our results showed significantly higher feed intake, egg production and egg yolk quality when the chicken were fed BSFL-based meal compared to the conventional feed. Mean egg production of layers fed BSFL-based feed significantly increased by 55.9% and remained economically viable for a longer period compared to those fed on convention feed. The implications of these findings for the layer chicken enterprise are discussed.

#### Behaviour and growth performance of young turkeys fed live black soldier fly larvae

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Black soldier fly larvae (Hermetia illucens) are a suitable protein source for poultry. However the effect of live black soldier fly larvae (BSFL) supplementation on growth performance and behaviour has never been demonstrated and quantified in turkeys. Wild turkeys eat insects during the first two weeks of life which is in contrast with commercially fed crumbs or pellets. Damaging pecking behaviour is a severe problem in turkeys. More lively diets may improve natural behaviour and decrease damaging pecking behaviour. The aim of the experiment was to stimulate natural behaviour of young non-beak treated turkeys by supply of live BSFL, and thus avoid damaging pecking behaviour. Two treatments with seven replicates were studied in 14 floor pens (1.5 m<sup>2</sup>/pen and 20 turkeys) from 0 to 35 days of age. Control groups were fed commercial diets and BSFL groups received live BFSL. The daily BSFL intake was calculated to be 10% of the expected daily feed intake (based on fresh weight) and dietary nutrient composition was adjusted in a way that control and BSFL groups were fed iso-nutritious. Daily feed intake and body weight gain of BSFL groups were significantly higher compared to control groups resulting in a significantly higher body weight at five weeks of age (2,190 vs 2,015 g; P=0.003) and a significantly lower feed conversion ratio. Feather and skin damage tended to be lower in the BSFL groups until three weeks of age and at 4 and 5 weeks a significant difference in favour of the BSFL groups was observed. In the first week there was a tendency for more foraging related behaviour for the BSFL groups and in the third and fifth week BSFL groups showed less foraging related behaviour compared to control groups. Provision of BSFL slightly reduced aggressive pecking directed at the back and tail base.

## Evaluation of carcass and meat traits of Muscovy duck fed with black soldier fly partially defatted meal

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The aim of this study was to evaluate the carcass characteristics and breast meat quality in Muscovy duck (*Cairina* moschata domestica) fed different inclusion levels of a partially defatted black soldier fly larva (BSF) meal. A total of 256 Muscovy ducklings (average live weight, LW: 71.32±2.70 g) were reared from day 3 to day 48 and randomly allotted in 32 pens (8 replicates/treatment). Four different diets were formulated with increasing substitution level of corn gluten meal with BSF larva meal (0, 3, 6 and 9%; BSF0, BSF3, BSF6 and BSF9, respectively) and divided in 3 feeding phases: starter (1-14 days), grower (14-35 days) and finisher (35-48 days). At day 48, 2 animals/replicate were slaughtered and dissected to determine their carcass yields. The weights of spleen, bursa of Fabricius, liver, heart and abdominal fat were recorded. Breast and thigh muscles were then excised from 16 ducks/treatment and weighted. Ultimate pH (pHu) and L\*, a\*, b\* colour values were then measured on breast muscle. The collected data were tested by means of oneway ANOVA evaluating the effect of dietary BSF inclusion level by polynomial contrasts. Significance was declared at P<0.05. The inclusion of BSF did not affect final LW (2,515.68±92.42 g on average). Hot and cold carcass weights showed a quadratic response (P < 0.05) to increasing BSF larva meal, with a minimum corresponding to BSF6; however, refrigeration losses were not affected by treatments. Weight of spleen, bursa of Fabricius, liver and heart did not differ among treatments. The weight of abdominal fat showed a quadratic response to increasing BSF meal with a minimum corresponding to BSF6 group (P<0.05). Breast and thigh yields, pHu and L<sup>\*</sup>, a<sup>\*</sup>, b<sup>\*</sup> colour values did not differ among groups. With the exception of BSF6, the inclusion of BSF meal did not affect meat traits and carcass characteristics, confirming the potential use of BSF meal in Muscovy duck diets.

## Ynsect production for the aquaculture sector: growth performances

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In 2015, Ynsect demonstrated the high quality and performance of its blockbuster product ŸnMealTM (Tenebrio molitor defatted protein meal) in juvenile rainbow trouts in comparison with a super prime fish meal 70 LT (+34% weight gain and -15% FCR after 90 days). Unpublished trials (for confidentiality reasons) on poultry and mice also showed significant impact on growth, well-being and the health of these animals. The company aims to diversify its market targets in many species and different regions. The white leg shrimp (Litopenaeus vannamei) is one of the main species in aquaculture, of which total production amounts to 3.7 million tonnes per year and where the largest producers are China, Thailand, Vietnam, and Indonesia. Ynsect launched a new trial with Kasetsart University (Bangkok, Thailand) on juvenile shrimps in 2016. The control diet contains 25% fish meal (FM) and a total of five different diets with increasing rates of inclusion of YnMealTM as a replacement for the FM, which were designed with iso-nutritive contents. No significant difference was assessed in the palatability test between the diets. The T5 diet (100% FM replacement by ŸnMealTM increased weight gain by 21% and final body weight by 12.4% after 8 weeks of feeding, but the best results were found for the 10.3% ŸnMealTM inclusion in the diet (50% FM replacement): an increase by 33.7% in weight gain and by 24% in final body weight after 8 weeks of feeding. The FCR decreased significantly by up to 25%. The apparent digestibility of proteins and lipids was above 97.4%. A challenge test was performed with a frequent pathogen in aquaculture (Vibrio parahemolyticus), responsible for the well-known early mortality syndrome (EMS). After 10 days, the survival rate reached 90% in the diet with 50% FM replacement by YnMealTM compared to 56.7% in the control diet. Mortality could be observed directly from 5% ŸNMEAL inclusion. The mortality was divided by up to 4, which is due to the patented1 bacteriostatic effect of ŸnMealTM and the constant increase of the phenol oxidase activity (up to +400% in the diet with 100% FM replacement by ŸnMealTM). Since the shrimp does not have an acquired immune system, this immuno-stimulant property is very promising.

## Apparent digestibility of insect protein meals for rainbow trout

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Insect meals are considered to be promising future ingredients for aquaculture feeds. In past feeding trials in rainbow trout, insect meals were included in diets only on the basis of their nutrients content and energy density without taking into account their biological availability due to the lack of their digestible values. Apparent digestibility (ADC) provides good indication of the bioavailability of nutrients and energy thus providing rational basis for the correct inclusion of feedstuffs. The aim of this research was to assess, in an *in vivo* trial on rainbow trout, the ADC of five full fat insect meals: one Tenebrio molitor (TM), two Hermetia illucens obtained through two different process (HI1 and HI2), one Musca domestica (MD), and one Alphitobius diaperinus (AD). Fish were fed a high-quality reference diet (R) and test diets obtained mixing the R diet with each of the test ingredients at a ratio of 70:30. Diets contained 1% celite as inert marker. Fish were fed to visual satiety twice a day and faecal samples collected using a continuous automatic device. Faeces were freeze dried and frozen (-20 °C) until analyses. The ADC of dry matter, crude protein and ether extract of each insect meal diet were calculated. ADC for dry matter varied between 70.07 (HI1) and 80.85 (TM). ADC for protein was above 84% in all treatments and resulted the highest in MD, TM and AD treatments. Ether extract apparent digestibility significantly differed among diets with the highest value reported for TM treatment. All treatments reported values higher than 96%. Observed differences could be due to the insect species and meal treatment but in general, tested insect meals were highly digestible for rainbow trout. The results from this research could be useful to optimize the diet formulation.

## Using insect meal as a sustainable protein source in tilapia feed

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Identifying safe, sustainable and affordable fish feed stuffs is a priority within aquaculture. Edible insect meal has been presented as a suitable alternative to fish meal and other protein sources for omnivorous fish. Insect meal would be of use within rural smallholder communities as farmers could rear the insects themselves. However, only few trials have examined the use of insect meal within fish diets and always within the concept of partial substitution of the fish meal component of the diet rather than as a standalone ingredient. As such a preliminary study was conducted to evaluate the efficacy and potential of insect meal as a standalone protein source for tilapia. Juvenile Egyptian Nile tilapia (*Oreochromis niloticus*) of the Abbassa strain were monitoring in outdoor nylon net cages over 12 weeks. Four diets were used within the trial. Two diets were control, one utilised a plant only protein source diet while the second utilised fish meal as a protein source. Two experimental diets were formulated, one using larvae of *Hermetia illucens* as a protein source and the other using adult *Gryllus bimaculatus* as the protein source. All diets were formulated to be nutritionally balanced. Tilapia were feed one of the four diets twice a day. Feed consumption and conversion efficiency, fish survival, general health and growth was measured over the course of 10 weeks. Results of this will be presented.

## Apparent digestibility of insect meals for Nile tilapia fingerlings

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In this study, nutrients and energy apparent digestibility coefficients (ADCs) from five insect meals (cinerea cockroach Nauphoeta cinerea (NC); Madagascar cockroach Gromphadorhina portentosa (GP); cricket Gryllus assimilis (GA); mealworm larvae Tenebrio molitor (TM) and giant mealworm larvae Zophobas morio (ZM)) were determined for Nile tilapia fingerlings. The study was carried out in the Fish Farming Station of the Federal University of Lavras, Lavras, MG, Brazil. A total of 900 fingerlings, 3 g initial weight, were distributed in 18,250-litre fibreglass tanks in a completely randomized design with 6 dietary treatments (control and 5 test diets) and 3 replicates tanks with 50 fingerlings each. ADCs were determined using a reference diet (control) with 0.5% chromium oxide ( $Cr_2O_2$ ) as inert marker and test diets that contained 70% reference diet, by weight, and 30% of the insect meal being evaluated. Fish were fed to apparent satiation twice daily for 15 days. Faecal samples were collected using modified Guelph system. Results were subjected to ANOVA and Tukey test using the software SISVAR 5.3. Differences were considered significant when P<0.05. TM and ZM had higher ADCs for dry matter (95.3 and 83.2%, respectively) compared to GP and GA (51.9 and 49.1%, respectively). GA presented lower ADC for crude protein (52.4%) than TM (88.6%) and ZM (81.1%). TM showed higher ADC for chitin (88%) than GP (80.2%) and NC (71.5%). NC presented the lowest chitin ADC among treatments. NC, GP, TM meals presented higher ash ADCs (86.9, 84.8, 78.6%, respectively) compared to ZM (52%) and GA (48%). The ADCs of gross energy were higher for TM (86.1%) and ZM (80.8%) than for GP (48%) and GA (43.1%). The digestibility results may provide useful information about insect meals nutrients and energy utilization by Nile tilapia fingerlings. The authors would like to thank Vidaproteina for providing the insect meals and Brazilian research funding agencies FAPEMIG and CNPq for financial support.

# Influence of black solider fly pulp on growth performance, body composition and health status of juvenile mirror carp

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The present study was conducted to investigate the effects of dietary supplementation of black solider fly pulp (BSFP) on growth performance, body composition and health status of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). A total of 270 juvenile mirror carp (14.51±0.03 g) were randomly allotted to five dietary treatments: a control group and four BSFP groups, in which BSFP was included at 25, 50, 75 and 100 g/kg, respectively. Then fish were fed to apparent satiation for eight weeks. The results indicated that the growth performance and body composition of experimental fish had no significant difference compared with the control group (P<0.05). The kidney index of experimental fish in BSFP groups were significantly higher than that of the control group (P<0.05). The activity of serum catalase of experimental fish in BSFP groups were significantly higher than that of control group (P<0.05). The activity of serum catalase of experimental fish in BSFP groups were significantly higher than that of the control group (P<0.05). The control group (P<0.05). Intestinal villus height, villus area and muscle thickness of BSFP100 was significantly lower than that of the control group (P<0.05). About intestinal digestive enzyme activities there were no significant differences in all groups. In conclusion, BSFP could be included into the diet to 75 g/kg of juvenile mirror carp without negative effect on growth. Furthermore, it could improve the non-specific immunity and function of intestinal tract.

## Histological evaluation in sturgeon's gut fed Hermetia illucens meal

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For many years fishmeal (FM) was the first protein source in carnivorous fish diets. Currently, FM is often replaced by vegetable protein meal (VM) even if high levels of substitution often lead to intestinal and hepatic damage that compromise animal health and reduce performances. Recently, studies have been conducted evaluating the effects of insect meals as FM replacer on performance, but only a few have investigated the histological effect. Moreover, no study has been done on sturgeon. The aim of this study was to evaluate the effect of inclusion of highly defatted Hermetia illucens (HI) larvae meal on liver and spiral valve histology of Acipenser baerii juvenile. Four diets were formulated: a control diet (HI0), in which FM was the main protein source, two diets containing respectively 18.5% (HI1) and 37.5% (HI2) of HI and a VM-based diet (CV) (with 49% of VM and without HI). At the end of the performance trial (data not shown), 12 fish per treatment were sacrificed by over anaesthesia. Samples of spiral valve and liver were taken, subsequently fixed by immersing in 10% neutral-buffered formalin and processed by standard paraffin wax techniques. Samples were stained with haematoxylin-eosin and Periodic-Acid Schiff. Liver was evaluated for hepatic degeneration on 12 samples. Morphology (length and integrity) and number of goblet cells, on 25 villi per 5 samples/treatment, were evaluated in spiral valve. Regarding hepatic degeneration, the Kruskal-Wallis test didn't show significant differences among groups; likewise, linear regression didn't show significant differences in spiral valve evaluation. At microscopical level, HI0 and HI1 spiral valves showed a better preservation of absorption epithelium and villus structure, and a lowest mucus quantity than HI2 and CV. The hepatic accumulation of lipids showed an increasing pattern among groups: lowest in HI0 and HI1, highest in HI2 and CV.

# The effect of defatted *Hermetia illucens* meal on growth performance and intestinal enzymes of gilthead seabream

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Shortages in fish meal and oil supply resulted in rising prices and in their substitution in formulated diets with nonoptimal effects on growth and animal health. Insects protein, especially black soldier fly (*Hermetia illucens*; HI) meal has recently investigated due to the valuable nutritional properties. This study evaluated growth performance and intestinal enzymes activities of seabream fed for four months with a control and three experimental diets containing increasing levels of HI meal. Fifteen fish per each treatment were processed at the end of the trial. Statistical evaluations indicated that the dietary inclusion of defatted HI meal in seabream fed up to 18.4% of inclusion level did not influenced any of the growth performance traits considered. On the other hand, HI inclusion level of 27.6% worsened the following parameters: specific growth rate, feed conversion ratio and protein efficiency ratio, while as far as the somatic indexes are concerned, fish fed the control and 18.5% HI diets showed the lowest hepatosomatic index values (1.31 and 1.30, respectively).

# Comparing whiteleg shrimp (*Penaeus vannamei*) larvae performance fed an insect meal-based feed to a commercial brand

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Global research is seeking to improve the efficiency of marine ingredients used in animal feeds. Interest for insects as an alternative source of protein to support the ever growing aquafeed industry has significantly increased over the last years. A premium black soldier fly (Hermetia illucens) larvae meal 'Entomeal' was used to formulate a shrimp larval test diet in which the animal protein was largely supplied by the inclusion of Entomeal. At these critical stages of development, shrimp larvae and post-larvae (PL) feed quality requirements are high to ensure optimum growth and survival. In a 15-day trial, the performance of whiteleg shrimp (Penaeus vannamei) larvae from mysis M1 stage to PL12 fed the test diet were compared to those fed a control commercial brand diet to assess the suitability and guality of Entomeal for shrimp juveniles. The trial was conducted within a reputable aquatic animal health laboratory in Thailand. Pathogenfree P. vannamei larvae at mysis M1 stage were allocated to 24×20-l tanks at a density of 100 larvae/l. Test and control diets were allocated to the tanks following a block design. Of the 12 replicates for each treatment, 10 were used for final data collection and statistical analyses, while the remaining 2 tanks were used as sentinels to monitor the progressive development of the stages during the trial and health status and to permit adjustments to the feeding programme. Sub-samples of each replicate were weighed at the start and on termination (80% of the sample PL from the sentinel tanks reached PL12) of the trial to determine the growth performance. On completion of the trial, PL survival was assessed and sub-samples from each tank were subjected to a standard stress test. Although not statistically significant, the shrimp fed the Entomeal-based diet had a higher average survival than the control (71.6 and 57.4%, respectively), which is critical in commercial production. The shrimp fed the test diet also had greater average muscle occupancy scores across PL2-11, of which 5 of the 10 daily assessments resulted in statistically significant differences; the final dry and wet weights of the shrimp were not statistically different. Significantly greater lipid vesicle number and gut fullness of the test batches were explained by the difference in feeding ration administered from day 9 (PL6) onwards. From the laboratory results and trial conditions used, it is concluded that *P. vannamei* larvae fed Entomeal-based diet performed at least on a par with those fed the commercial diet. The quality performance of the PL, which are critical stages of development, highlight the high quality of Entomeal and suggest a wider potential of application in aquafeeds.

#### Conceptual model of biowaste processing with black soldier fly larvae

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Black soldier fly larvae (BSFL) are efficient converters of biowaste into animal biomass for feed production. The sustainability of insect feeds mainly depends on the substrate used for primary production. The use of municipal organic solid waste has a high potential of environmental benefit. However, these biowastes are highly variable in their nutrient and microbial composition. This poses a challenge for safe and efficient treatment and BSFL feed production. Based on available literature of fly larvae important in forensic, medicine and genetics and laboratory experiments with BSFL we present a conceptual model for the biowaste digestion strategy of BSFL. Reviewed fly larvae include Musca domestica, Lucilia spp., Stomoxys calcitrans and Drosophila melanogaster. Laboratory experiments included production of sterile larvae, determination of gut dimensions, diet residence time and gut pH. BSFL development is variable between substrates. BSFL gut physiology suggests that one reason for this variability is the different digestibility of biowaste nutrients. BSFL have a short for- and hindgut and a large midgut. In comparison to other insects with a complex hindgut digestion, this suggests that only nutrients with a high digestibility (e.g. simple sugar, proteins) or that have been pre-digested by microorganisms can be absorbed in the midgut for growth. This suggest that process efficiency could be boosted by the design of biowaste pre-treatments, e.g. for dietary fibres that have a low digestibility. Microbial dynamics in BSFL production and feed safety are a major concern for the currently growing industry. The acidic (pH: 2-4) anterior and basic posterior (pH: 8-10) midgut sections suggest that selective inactivation of microorganisms occurs within BSFL depending on strain, diet, larvae density and microbial dose. Short biowaste residence times of several hours suggests further that some microorganisms cannot multiply fast enough to be retained in the larvae. This is important preliminary data for integration into existing digestion and microbial community models (e.g. humans, other animals). Thereby, it could contribute to better understanding microbial dynamics during BSFL production and design of effective processing technologies for safe feed production.

#### Implementation of circular economy through black soldier flies for non-food applications

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Danish Technological Institute (DTI) has been working on bioconversion of organic waste products since 2012. Initially, this work was with the housefly (Musca domestica) on chicken manure, but during the last couple of years DTI has focused their efforts on black soldier fly larvae (BSFL) as main 'tool' for converting organic substrates. Currently, DTI is part of a handful of national and international R&D projects involving pilot production with BSFL reared on organic household waste as well as assessment of the potential of other high volume residuals as feed. Overall, our work with insects, and BSFL focusses on implementation of circular economy – where insects have a great potential to contribute with technological solutions and products. In this presentation, we will show some of the results that have been generated on BSFL conversion of a variety of different substrates – this will include: (1) Optimization of feeding amount and frequency using pulped organic household. Large differences were observed in production time and utilization of feed even within minor variation in feeding amount and frequency. This clearly underlines the importance of thoroughly examining production parameters. (2) Biomass yield and feed conversion efficiency based on mass-balance data from a 6-month pilot production using pulped organic household waste. During the pilot production, more than 1 tonne of waste has been converted and the influence of seasonality in waste composition and larvae density has been investigated. A feed conversion factor of approx. 2 (dry matter feed to dry matter BSFL) was achieved in majority of the pilot production. (3) Assessment of BSFL converted by-products (compost) as fertilizer and as substrate for biogas production. This aspect is often neglected; however, to assess the economic viability and environmental impact it is important to include all the products from the production. (4) Digestibility and palatability feeding trials with BSFL (inclusion from 0-13.5 ww%) in mink. Protein and fat was digested at 86 and 90%, respectively; equivalent to the digestibility of fish-meal in mink. Assessment of palatability of BSFL in mink was also very promising ranging from 96 to 99% of all the feed being consumed. In the presentation, preliminary data from a full-scale mink feeding trial will also be included with emphasis on processing and the effect on digestibility and skin quality.

## Efficiency of organic stream conversion by black soldier fly larvae: Session 8 a review of the scientific literature Processing organic side streams 3

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There is a growing interest in black soldier fly (Hermetia illucens, BSF) larvae as converters of low-value organic streams into a high-value proteinaceous feed source with an expected low environmental impact. As such, BSF larvae can increase the availability of feed proteins and mitigate the demand for unsustainable soybean and fish meals. The characteristics of the organic streams play a pivotal role in this concept as well how efficiently these can be converted into insect biomass. Our objective was to investigate larval conversion efficiencies for tested streams as described in scientific literature. Articles published in peer-reviewed scientific journals before September 1, 2017 were retrieved from online databases (Scopus, Google Scholar) and additionally via checking the reference list and citations of each article. As varying moisture levels of streams (12.3 to 31.7%) and larvae (17.9 to 38.8%) were reported, only data on dry matter (DM) and N basis were included in the review. Forty articles evaluated in total 78 (mixtures of) streams. For these studies, experimental procedures varied in terms of feeding ration and regime, larval age at start, harvest stage and measured parameters. Furthermore, chemical characterization of studied organic streams was in general limited or absent. Conversion efficiency (insect biomass collected divided by feed provided in %) on DM and N basis was reported or could be calculated for 21 and 13 streams, respectively. Conversion efficiency of DM for control feeds (i.e. poultry feed, Gainesville housefly diet, processed wheat) was 10 to 23% whereas this was 2 to 63% for the streams. Nitrogen conversion efficiency values were 24 to 54% (controls) and 7 and 75% (streams). It is concluded that BSF larvae can thrive on a wide range of organic streams, but DM conversion efficiency is known for less than 25% of the streams studied. Standardization of methodology in terms of chemical characterization of the organic stream used, rearing methods and post-harvest analyses is crucial to assess the potential of BSF larvae to convert such streams and to improve our understanding of factors important for efficient conversion.

## Side streams of Finnish bio industry as possible insect feeds

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One of the greatest global challenges of today is to sustainably provide the world's growing population with a secure supply of nutritious food, using fewer inputs and land. To meet the current and future challenges of food and nutrition, the means of how food is produced needs to be re-evaluated: inefficiencies must be identified, food waste should be reduced, side-streams should be re-used, and novel innovative methods to produce food need to be developed. Insects as food and feed are now considered as one potential solution to meet the increasing global demand of food and sustainable food production since insects have the capacity to utilise organic side stream materials. This study aimed to evaluate the development and growth of two common edible crickets Acheta domesticus and Gryllus bimaculatus fed with cricket and chicken feeds where soy protein was replaced by plant based Finnish bio industry by-products. We used Patton's diet no 16 for crickets, that has been published as being optimal, and commercial chicken feed as control diets. The protein source of the control diets was soy protein. In the experimental diets, the soy protein was replaced with residue potato protein, compressed leftover of turnip rape, barley mash, barley or broad bean and pea that were side streams of bio industry. The experimental units were placed as a randomised block design. There were five blocks per species and every block included two replicates of each diet treatment. The control or experimental diets and water were provided *ad libitum*. The crickets grew well with different side stream feeds. The best diet treatments were barley mash and potato protein that produced the heaviest individuals and highest survival on two different protein levels for both species, A. domesticus and G. bimaculatus. We concluded that side stream by-products have a great potential as insect feeds since insects accept, utilize and successfully develop with varying plant based materials. The use of side streams as insect feeds advances the strategies and targets of the circular economy.

# Turning Ugandan waragi waste into value-added livestock feed using Hermetia illucens

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Waragi, a form of homemade gin, is produced throughout Uganda in both legal and illegal breweries. In the processes of brewing waragi a molasses-esque waste product is produced. This is disposed of by direct dumping onto the ground surrounding the brewery or by pouring into nearby rivers. This waragi waste has been documented by local communities to be toxic to plants and animals. Government efforts to decrease the amount of waragi brewing have been unsuccessful as it is a vital source of income for vulnerable communities. As such it would be beneficial to the community and environment if a use could be found for the waragi residue. *Hermetia illucens* larvae are documented to consume a wide range of otherwise unappealing waste products, including faecal waste. The larvae then serve as a high-quality protein source for livestock; namely chicken, fish and pigs. A dietary toxicity trial was run to establish an  $LC_{50}$  value for waragi inclusion. This was followed by a larger scale trial utilising waragi combined with various *in situ* available feed stuffs to further assess the viability of processing waragi waste using *H. illucens*. Growth rates, size, feed consumption and survival were monitored daily over two weeks. Results of this and future implications are to be presented.

# Learning by doing – experiences with a pilot BSF waste treatment unit in Indonesia

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A fairly novel approach is rapidly establishing itself and concerns urban biowaste conversion by larvae of the black solider fly (BSF), Hermetia illucens. The popularity of this approach links to the promising opportunities of using the harvested larvae as protein source in animal feed thus providing a valuable alternative to conventional feed components that require a large amount of increasingly scarce resources (water and land for soya meal, and fish catch for fishmeal). The project presented here has developed a pilot treatment site in Indonesia, at a vegetable/fruit wholesale market. Now a decentralized urban waste treatment system, which is a modular manually operated system build-up of crates and frames increasing the waste treatment capacity of a waste transfer station, is being implemented. A centralized nursery ensures a stable supply of young larvae and a centralized post-processing plant can ensure a higher quality product through combining the harvest from each satellite waste treatment unit in a centralized product refining process where more advanced equipment can be used to fraction the larvae and produce pure raw materials. A trial phase treating 13 tonnes of source-segregated biowaste from a city district was started in June 2017 and completed in January 2018. The FORWARD project managed to train a team of five waste handlers, previously working in the compost facility, on processing waste using the simple modular BSF waste treatment units. A basic data collection system was implemented for the waste transfer station to continuously monitor the performance of the waste treatment units and to manage their production of BSF larvae. Some research questions however still must be answered to best promote this treatment option as an attractive biowaste treatment approach for waste managers and businesses: (1) Although feed trails with BSF larvae have confirmed the suitability as alternative for fishmeal, more insights on associated risks linking waste feedstock quality to larvae (e.g. feed) quality are required. (2) Waste reduction up to 80% on wet weight basis has been demonstrated but knowledge on how to (post-) treat and market this residue as a product is still lacking. (3) Results confirm that there is no need for a sophisticated high-end technology to operate such a facility. However, field experience from Indonesia show that the main bottleneck lays in obtaining source-segregated household waste. (4) Optimizing the space footprint and reducing climate emissions continues to be 'work in progress' especially in urban dense settings and climate mitigation policy priorities.

# Bio-conversion of three organic wastes by black soldier fly (*Hermetia illucens* L.) larvae in New Zealand

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Larvae of the black soldier fly (*Hermetia illucens* L.) are of great importance in organic waste management, because of their ability to voraciously feed on various types of organic waste. The larval biomass resulting from organic waste bio-conversion is rich in protein and fat, and thus can be used as a novel feed ingredient for aquaculture, poultry, and livestock. In the present study, we investigated the potential of using black soldier fly (BSF) larvae to establish a novel value-added organic waste management system for local businesses in New Zealand. Three types of organic wastes, brewer's waste, solid phase of pig manure, and deer paunch waste, were selected based on current waste management approaches in relevant businesses in the country, such as landfill and compost, and the resulting economic and environmental costs. We tested the suitability of using BSF larvae to convert the target organic wastes into insect biomass, in comparison with a standard larval diet based on wheat middlings. Larval development and weight gain, nutritional composition of larval biomass, waste reduction ratio, and bio-conversion efficiency were measured. The results revealed that brewer's waste was the most suitable waste to be bio-converted by black soldier fly larvae, while deer paunch waste was the least suitable. The possible underlying physiological adaptations of BSF larvae in response to the nutritional composition of the organic wastes are discussed, as well as several approaches to increase waste reduction ratio and bio-conversion efficiency by BSF.

# Attenuation of antibiotic resistance genes in chicken manure by black soldier fly larval conversion

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Antibiotic resistance genes (ARGs) in animal manure contribute to the increase in multidrug-resistant pathogens in the environment and are a hazard to public health. The bioconversion of manure with fly larvae is a promising alternative of recycling animal wastes while reducing the abundance of ARGs. We investigated the impact of black soldier fly (BSF, *Hermetia illucens*) larvae bioconversion of chicken manure on the persistence of AGRs. Compared to traditional compost methods, BSF larvae reduced absolute abundance of ARG and integrin genes by 95%. This reduction was in part due to rapid decreases in concentrations of the genes and of bacteria as they passed through the BSF larval gut. Consequently, bacterial community composition differed significantly with the percentage of Firmicutes possibly carrying ARGs reduced by 65.5% or more. We suggest selective pressures imposed by microbial exposure to the alternating manure and larval gut environments and larval bacteriostasis contribute to ARG attenuation.

## Almond hulls - a good feedstock for insect farming

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California's central valley, with a Mediterranean climate, provides optimum conditions for almond production. In 2017 California almond industry produced 1,017,000 metric tonnes of almond kernels from 454,500 hectares of bearing orchards. California is the only state in the United States where almonds are commercially grown, now producing more than 80% of the world's supply. With 35% of the volume consumed domestically, the industry exports 65% to more than 90 countries. China has been the second largest export market for several years. Almonds grow in a shell, protected by a hull, on trees. Almond hulls representing over 50% of almond biomass are 12-14% moisture, with up to 62% dry matter water extractable including over 30% fermentable simple sugars. The majority of non-extractable compounds from spent hulls are cellulose, hemicelluloses and lignin. The hulls have historically been sold as a feed supplement for the California dairy industry. As the California almond industry continues to grow in production, there is an ongoing challenge to find more outlets for the co-products. The Almond Board of California (ABC), representing all almond growers and processors in California, has been charged with finding more value-added solutions and expanding feed applications and markets. ABC has been collaborating with China AQSIQ to obtain market access for almond hulls as feedstock with an expectation of a final approval by 2019. ABC has funded several research projects to improve feed value of almond hulls. The preliminary feeding studies of black soldier fly larvae with almond biomass at University of California Davis have shown very promising results. This presentation will cover the resources and characteristics of almond hulls as a feedstock for novel feed applications.

# Impact of soybean curd residues co-conversion by Hermetia illucens larvae assisted by Pedicoccus acidilactici on biomass

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Black soldier fly larvae (BSFL), *Hermetia illucens*, develop on organic wastes, reduce ecological pollution and convert wastes into protein and fat rich insect biomass. The relationship of nutritional characteristics and food safety in BSFL during its development on organic wastes is still poorly understood. In order to evaluate the conversion of soybean curd residues (SCR) into high quality animal derived proteins and fats for human and livestock feed consumption, this study assessed co-conversion efficacy, nutrition composition, safety and anti-nutritional factors concentration in BSF after developing on SCR and *Pedicoccus acidilactici* The soybean curd residues were pretreated with *P. acidilactici* ( $10^8$  cfu/ml) and afterwards BSFL were employed for conversion. When the larvae were fed with SCR and *P. acidilactici* ( $1.3^{-9}$ ), dry mass reduction ( $55.70\pm0.93\%$ ) from the substrate, bioconversion ( $6.95\pm0.31\%$ ), feed conversion ratio ( $8.01\pm0.31$ ), protein content ( $55.26\pm0.62\%$ ), and fat content ( $30.00\pm0.61\%$ ) were significantly increased when compared with SCR and artificial feed. The amino acid and fatty acid profiles developed on *P. acidilactici* assisted BSFL showed satisfactory level of all essential amino acids and fatty acid required for human consumption as directed by WHO/FAO/UNU. In addition, heavy metals and anti-nutritional factors concentrations showed very low value and were observed within the safety intake level for human and animal nutrition. Therefore, the addition of *P. acidilactici* with BSFL on SCR does not only increase the co-conversion process but it also enhances the nutritional values in BSFL.

## Farming crickets using food waste: an action-based approach

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Amidst the global ecological crisis and a growing world population, the need for sustainable food systems is more important than ever. Using insects as food is suggested as a means to secure high-quality nutrition at low environmental costs. The farming of insects is referred to as mini livestock, and the cricket species Gryllus bimaculatus de Geer 1773 is a popular species for farming. Being an omnivore, this cricket species can be fed using various types of organic side streams, including food waste. In addition to potentially lowering the production costs of cricket farming, this practice constitutes a form of nutrient cycling. However, the nature, handling, and availability of local organic side streams is complicated and unclear. Furthermore, while there is a history of eating crickets in Taiwan, this practice has become practically obsolete. Finally, what equipment and knowledge are needed to engage in cricket farming? In this study, action research was used as a methodological framework to investigate these distinct areas. The approach allowed producing local knowledge, facilitating experiential learning, and creating the conditions for continuing action. For 11 months, successive cohorts of G. bimaculatus were reared on local food waste. Observation and survey work in the local vicinity allowed understanding the handling of food waste by actors in the food and beverage sector and estimating the nature and amount of available food waste. Crickets were harvested, prepared as food and eaten with various participants. The action research approach was the foundation for continuing and scaling up the farming of crickets as well as widening the circle of public participation and awareness raising. The findings revealed that G. bimaculatus can be successfully reared on food waste. However, most local food waste is already used privately or given to farmers. The locally available food waste could support between one and nine small-scale cricket farms producing between 15 and 23 kg fresh weight per rearing cycle. When whole crickets were presented to participants as food, personal involvement of the researcher, conversation regarding the rearing as well as the topic of edible insects in general, and demonstration (of eating them) was associated with higher acceptance. Finally, the subtropical climate of Hualien, Taiwan is not suitable for all-year unheated rearing. Three to four harvests are conceivable during the warm months. In winter, the cold temperatures mean that cricket development is unfavourable for farming.

#### Techno-economic assessment of insect ingredients production

#### Session 9 Nutrition, processing and conservation 1

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The rapid development of modern society has resulted in an increased demand for food supply, and more particularly that of protein. In this context, rearing insects for food and feed by transforming organic side-streams may be an effective solution. This work consists of a techno-economic assessment of a mealworms transformation facility. Six different scenarios were considered. First of all the size of the production infrastructure: (1) artisanal scale – 500 T flour/year from 1,900 T mealworms/year; (2) industrial scale – 10,000 T flour/year from 38,000 T mealworms/year. Then the quality of mealworm flour: (F) flour, (DF) defatted flour and (PF) protein-enriched flour. This analysis has demonstrated that the economic viability of the six scenarios is quite dependant of: (1) processing methods; (2) the type of equipment; and (3) the selling price of flours and purchase price of mealworms. This analysis has demonstrated that the economic viability of the six scenarios is quite dependant on: (1) processing methods; (2) type of chosen equipment; and of course (3) the selling price of flours and purchase price of mealworms. For all the facilities, the net value- added decreases considerably when producing PF. For the artisanal facility, only producing F and DF is an economically viable process.

# Drying of insects and larvae: towards valorisation into high-value functional proteins

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The current global context of food market and protein consumption is dominated by a degradation of the environment, an increase of the density of population and a rarefaction of the agricultural and natural resources available for their productions. In spite of the deep interest for insects, as natural resources rich in proteins with high efficiency on their productions, scientific and technological steps have to be raised before to be incorporated as protein ingredients in human food formulation or even in animal feeds. Concerning transformation, insects must undergo a series of treatments in order to make them fit for industrial use. Of utmost importance is the need to master the quality and supply of the raw material (selection, development stage, insect diet), as well as operating conditions necessary to obtain end-products showing the desired features. The drying process is a necessary step to stabilize the fractionated insects or to standardize them before industrial treatment. However, the drying step is often a real bottleneck for the whole production line. This limitation of productivity, combined with the decisive impact on the quality of the dried insects, requires research and development efforts that are still substantial. The drying process has also the particularity of being one of the strongest consumers of energy, because it is carried out in the majority of the cases by the thermal way. It is therefore essential to optimize the operating conditions of drying in order to obtain a better quality of the finished product and a high-energy performance. For example, the control of the drying temperature is important for thermosensitive products such as insect larvae. High temperature may improve the dryer efficiency (ratio of the heat of evaporation to the heat put into the dryer), but may cause also thermal degradation or burning of the drying product. The overall objective of this study is (1) to conduct experimental characterization of the drying of pressed larvae using a lab scale rotary dryer, and (2) to develop a mathematical model for a co-current rotary dryer, to predict the temperature and the moisture profiles of the drying gas and the feed pressed larvae along the length of dryer. Ultimately, this study will provide a basic tool for simulating the drying of insect larvae and for optimization purposes.

# Insects: protein content and browning/blackening during processing

Session 9 Nutrition, processing and conservation 3

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Insects have been identified as an excellent source of protein. The acceptance of insects increases when used as ingredient in an invisible manner and hence grinding is often performed. Browning occurs upon grinding insect larvae, which could hamper their potential use as ingredient for food and feed. The aim of this work was to investigate the specific nitrogen to protein conversion factor (Kp) to determine accurately protein content, the mechanisms responsible for the browning or blackening of insects during grinding, and its impact on protein functionality. Three insect species were studied, Tenebrio molitor, Alphitobius diaperinus and Hermetia illucens. The specific Kp factor of 4.76±0.09 was determined for the three species. After protein extraction and purification Kp was  $5.60\pm0.39$ . So, the general Kp factor of 6.25 overestimates the protein content in insects. The browning upon grinding was found to be caused by both enzymatic and non-enzymatic browning for all three insect species. Phenoloxidase (PO) was mainly responsible for browning in T. molitor and likely in A. diaperinus, whereas the presence of iron most prominently contributed to the dark black colour in H. illucens, likely by iron-phenolic complexation. A model system of L-DOPA and iron was used to elucidate the structures of the iron-L-DOPA complexes. Enzymatic browning did not influence the solubility of the proteins of all three species by using sulphite as PO specific inhibitor yet the solubility was compromised after blanching (50 s, 90 °C). Upon in-vitro hydrolysis by pepsin and trypsin, soluble proteins from *H. illucens* were more digestible compared to T. molitor and A. diaperinus. Phenoloxidase activity during processing negatively affected in-vitro pepsin hydrolysis. Next to phenoloxidase activity, also endogenous proteases remain active at pH 8 in insect extracts. The endogenous enzyme activities and iron complexation should be taken into account for future application, as well as the specific Kp factor to prevent overestimation of the protein content of insects.

# Comparison of nutritional composition of five commercially available edible insects and honeybee brood

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We evaluated nutritional potential and functional properties of five commercially available insects namely Allomyrina dichotoma (Coleoptera: Dynastidae), Protaetia brevitarsis (Coleoptera: Cetoniidae), Tenebrio molitor (Coleoptera: Tenebrionidae), Teleogryllus emma (Orthoptera: Gryllidae), and Gryllus bimaculatus (Ortoptera: Gryllidae) used as food and animal feed and honeybee brood Apis mellifera ligustica (Hymenoptera: Apidae). Chemical analyses were carried out following the standard method of AOAC. Most of these species contained high protein contents with low variation (53.2 to 58.3%) except for *P. brevitarsis* and honeybee brood. Fat contents of the insects varied widely (11.9 to 34.5%). T. molitor contained the highest amount of fat (34.5%), suggesting a possible future role as a commercial source of oil. A total of 17 amino acids (8 essential, 1 conditional essential and 8 non-essential) and about 26 fatty acids (12 SFA, 6 MUFA, and 8 PUFA) were determined. Except for methionine, all other essential amino acids satisfied the protein level recommended by FAO/WHO/UNU. The MUFA proportion was highest in the beetle larvae, but PUFA contents were maximal in the cricket adults. However, PUFA was absent in honeybee brood although a trace amount was found in the adult bee. Compared with conventional animal meats and chicken eggs, especially the crickets that we examined seem superior from a nutritional perspective as they contain higher amounts of protein, iron, zinc, and magnesium and possess fats with fewer SFAs but more PUFAs (with the exception of A. dichotoma and bee brood). The paper also demonstrated the anti-oxidant properties, free radical scavenging activities of the insects. The inclusion of bee brood, of course to a certain extent, in the list of hive products, could also improve the economic situation of small and medium scale beekeepers. Systematic farming of these insects could, therefore, be a choice in the quest to replace vertebrate animal food products with a sustainable alternative.

# Amino acid composition and protein quality of Eri-silkworm (Samia cynthia) pupae flour

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Food provision for a billion people is a challenge, especially in terms of animal protein. Eri-silkworm or *Samia cynthia* is one of the wild silkworm species which consumes castor leaves (*Ricinus communis*) as the main feed. The pupae are rich in nutrients, mainly protein. The aims of the study were to analyse the amino acid composition and protein quality of Eri-silkworm pupae flour. The amino acid composition was analysed by using ultra performance liquid chromatography (UPLC). The highest essential amino acid content in the Eri-silkworm pupae flour is the 78.25 mg/g aromatic amino acid and the highest non-essential amino acid is the glutamic acid of 84.54 mg/g protein. The protein quality was determined by the chemical score (46.18 for sulphuric amino acid), the amino acid score (72.69), the essential amino acid index (EAAI=0.99), the predicted protein efficiency ratio (P-PER=1.65), and the predicted biological value (P-BV=45.50). The study showed that Eri-silkworm pupae flour contain high-quality protein.

# Effects of domestic cooking on protein digestibility and mineral bio-accessibility of wild harvested edible insects

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Consumption of wild edible insects is still prevalent and common in both rural and urban areas of Zimbabwe. Henicus whellani and Eulepida mashona are such two wild insect species consumed. A previous study on the nutritional composition of raw insects showed that they have high protein and mineral content. A need exists to evaluate how domestic processing influences the nutritional composition to get a clear picture of the extent the consuming populations are benefiting from the insects nutrients. Therefore this study aimed at determining the influence of domestic processing on the protein digestibility and mineral bio-accessibility of *E. mashona* and *H. whellani*. Samples of both insects were subjected to boiling, roasting and combined boiling and roasting, imitating way the insects are prepared traditionally. Protein and mineral content loss or retention were assessed. In vitro digestion of the samples was done according to Infogest protocol. OPA and ICP-AES analysis were performed to determine the soluble protein and mineral fraction after digestion respectively. Protein digestibility was calculated based on the total hydrolysis method according to Schasteen *et al.* Protein digestibility of both raw insect species before processing, were comparable but lower than of whey proteins (35%). Boiling and roasting resulted in reduced protein digestibility, the reduction was dependent on the time of boiling. E. mashona had a higher iron bioaccesibility as compared to H. whellani. Zinc in E. mashona was more accessible than iron. Boiling and roasting resulted in decrease in mineral bioaccesibility. Low protein digestibility can be attributed to chitin or protein conformation binding to other food matrix components. Reduced digestibility and accessibility with processing can be explained by protein modification and presence of phytochemicals. Because of the reduced protein digestibility and mineral accessibility due to processing, minimal boiling should be done. By so doing the potential of the insects to nutrition security can be improved.

#### Mealworm larvae production systems: transformation processes – insect-based protein products

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Satisfying the growing demand for proteins, notably for the purpose of feeding animals, and toning down the impact of animal husbandry on the environment, while at the same responding to public demand for product quality, are major challenges for international organisations, private operators and agri-food researchers. Among several possible additional sources of proteins, the insect solution seems to be relevant and credible as a complementary option to other conventional ones (fish and soya). The development of an insect industry must necessarily take into account the availability of the resource, rearing methods as well as transformation technologies. But one must also keep in mind the potential and accessibility of markets targeted. The question of the choice of a particular transformation process, and the resulting quality of products is crucial to satisfy the needs of users (some expect insect flour, insect-protein isolates or hydrolysates). However, systematic studies assessing critical unit operations of insect protein recovery procedures are scarce. This presentation will focus on a specific a part of the work carried out within the framework of the DESIRABLE project ('DESIgning the Insect bioRefinery to contribute to a more sustainABLE agro-food industry'), funded by the French National Research Agency (ANR). The ambition of this project was to cover an entire new insect-based valuechain, from the feeding of insects and the transformation into protein-rich materials, to the final consumption in poultry and fish farms. We present different processes firstly to produce mealworm flour with larvae, and secondly to quantify their respective flows of energy and matter. The flours obtained are of two different types, whole meal flour and one that is partially defatted. Results have been obtained from selected pilot-trials and from interaction with equipment suppliers. Sufficient data have been generated in order to conduct a preliminary evaluation of the technical feasibility and the economic relevance of the transformation of insect larvae (Tenebrio molitor) into flour. The actual needs of the fish-farming and poultry industries have been taken into account. The chemical composition, the amino acid profile and techno-functional properties of insect-based protein products have also been taken into consideration. It is hoped that these results will contribute to a better understanding of *T. molitor* industrial processing systems.

# Nutritional contribution of insects to improve diets in Kenya: from known to unknown

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Insects can improve dietary quality by supplying protein, fat, and key micronutrients. However, nutritional composition of insects is highly variable within and between species of significance to human diets. Variation could in addition be caused by gender, life stage, diet, and environmental conditions. Lack of standard analytical methods specifically adapted for insects further complicates the variation. Moreover, bio-availability of most of these nutrients is often scanty or unknown. Influence of fractionation, extraction of bioactive compounds and processing on the nutritional quality is also not explored. Interaction of nutrients with common industrial additives is also unclear thus reducing industrial application of insects in the food industry. For insects, food neophobia is clearly high and this may affect their nutritional potential. To reduce insect food neophobia, studies suggest to insert invisible insect in food preparation and/or to associate them with known flavours. There is therefore need to initiate activities that will continue to answer some of the gaps that may hinder exploitation of insects in the food and nutrition sector.

# Can cricket-based porridge improve nutritional status of school children in Kenya – a randomized controlled trial

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To access the nutritional impact of cricket based porridge on child nutrition, a randomized controlled trial was set up. For the study, 138 children age 3-5 years were recruited. Children were given a daily serving of porridge for 115 days, either randomized to: intervention cricket porridge (5% cricket); positive control (10% skimmed milk powder) or negative control maize-millet (no insect/milk). Multi-micronutrient premix, 2% soy bean oil and 5% sugar were equal in all groups. All children improved nutritional status over the intervention period. Crickets have the potential to improve school feeding programs in Kenya.

#### Sustaining nutritious diets for Cambodian children with crickets

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Appropriate, quality foods may be inexpensive and locally available, yet are often under-used, with resulting high levels of malnutrition among infants and young children. Entomophagy is a traditional practice in 113 countries, including Cambodia. Crickets are a rich source of both macronutrients, and micronutrients, thereby making them an ideal complementary food to help achieve optimal growth. When exposed to cricket powder in typical porridge, taste tests found 95% of caregivers liked the food. Crickets are amazing! Key growth-nutrients are exceeded by locally-grown funky foods as compared to more expensive animal sourced foods. Through homestead production of quality crickets, this project enhances sustained access to and consumption of crickets among this very vulnerable age group (children 6-23 m). The objective is to sustain access to cricket powder through homestead production. Promoting sustainability of nutrition-rich food sources for families will ensure continued access to this nutritious food powder. Optimal feed preparation for quality cricket powder will be researched. Community nutrition workers and families be trained to grow, harvest and safely preserve micronutrient-rich crickets. Inexpensive solar dehydrators will be tested to prepare crickets for grinding into powders. Contests will be held to mobilise communities to create cricket cakes, cookies and chips. Progress (growth) will be monitored and results showcased. New communities will manage cricket production. 60% families raising and processing crickets; 50% children consuming crickets; 40% children gaining weight. The simple methods of raising and preserving crickets properly in inexpensive containers, access to cricket foods will be maintained in the long-term. Caregivers will share their results (healthier children) will their neighbours, thus improving the community norms around nutrition practices. Homestead production of crickets can be used to create a revolving fund to expand to other families, and other communities.

#### Edible insects under all its forms: from niches to mass market?

Session 10 Marketing and economics 1

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Knowledge and acceptance of insects as a food source grew generally in Western countries during the past few years. With some difference between countries, a rising attention from media, institutions and scientists led spread the idea that edible insects can be a part of our diet. What can we, today, for turning 'Insects, the food of tomorrow' as a today's food? JIMINI'S was created in 2012 in Paris by Bastien Rabastens and Clément Scellier in order to 'foodicize' insects in Western countries. To achieve this long and challenging mission, a strong emphasis was put on 3 aspects: (1) An adapted food product with a great attention on taste, nutritional value, packaging and everything adapted to the consumption moment; (2) The educational aspect with a focus on the mission, transparency and necessary information; and (3) A strong partnership with European insect breeders and a control on the food supply chain, from R&D, processing, logistics, marketing and communication to sales. To make insects 'exceptionally normal', the first range of products was seasoned insects as appetizers that are now followed by a whole range of products. To develop a first food product as appetizer seemed to be the more appropriate. It is a particular moment in France, where people are more eager to try and share new foods, where minds are opened and discussions easier. In order to reassure the consumer, a great effort was put on the packaging. The goal was to make it unique, put the insects forward and provide all the necessary information to make the consumption more engaging. In order to familiarize the consumer, the first step was to adapt the product for the French palais. Once the visual barrier is broken, the other organoleptic factors are already well-known. More than simply introducing a food, a whole consumption scheme needed to be created. In order to turn the 'insect' into an 'appealing food', our personal experience showed that using a light tone was the best way to break the first barriers. In order to not lose track of the goals and issues that edible insects can tackle, we decided to collaborate with schools and events to educate today's and tomorrow's consumers. Those strategies allow more than 1 Million of people across Europe to 'jump in' and try edible insects. With a more experienced industry and a field that starts getting shaped, will those strategies allow edible insects to be sustainably and durably integrated into our diet? In a multitude of 'Western diets', what will be the strategies to get through the 'trend' effect? That will be the challenge of our industry for the next years.

## Bug based packaged food: market obstacles and opportunities

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Edible insects have been talked about on Western media for over three years now but are there products on the supermarket shelves? Despite the efforts of a number of start-ups and associations, there are still a limited number of packaged food based on processed bugs. And the mainstream distribution does not seem to believe these products can move fast. Analysing the commercial obstacles and opportunities is critical to demonstrate that edible insects can really turn into an industry. Regulations (or the absence of rules) are also slowing down the market. The problem is not limited to Europe, with the new Novel Food law, but also to other worldwide guidelines and legislations. For example there are no product codes for insects, according to the Worldwide Custom Organization, which makes import and export more complicated. And it will takes years before insects will be included in the FAO's Codex Alimentarius. Even countries where insects are commonly eaten are often considered illegal. In China for example, only the silkworm is listed as a legal food. How do we get products into the mainstream market? Are cricket energy bars the way to start from a niche and then move to the average consumer? Is the mealworm having better chances than the cricket, considered the gateway bug for the last two years? Is silkworm the Chinese third way, since it represents 500,000 tonnes produced yearly in China? The presentation will try to give an answer to these key commercial questions.

#### Automation vs labour: consequences of production decisions in scale insect farming

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Insect wild crafting, cultivation, and farming have a long history, but only recently have sophisticated modern operational methodologies been applied to this industry. Automation has the benefit of efficiencies at scale yet presents significant drawbacks. The up-front capital costs of modern automation are not trivial and put scale application out of reach for many. Even when not cash-constrained, technical hurdles can prove more challenging than expected, and solutions that work at the bench-top level often break down at scale. Manual techniques have the benefit of low capital costs, ease of organic scaling, and viability from bench-top to massive facility. The key draw-back of manually intensive processes is the inability to leverage true economies of scale. This talk will discuss a range of different approaches to insect production at scale, explore the costs and benefits of each, and present recommendations for new entrants and existing parties in the industry.

## Insights from marketing edible insects and feed insects in South East Asia

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Insects both in food and feed have gained significant global momentum and there is a rising public awareness of the existence of food and feed applications for insects among South East Asian consumers and businesses. While there has been an increasing body of research on customers for food and feed insect products in Europe and North America and references to the 'traditional consumption of insects in South East Asia' are regularly made, little research on the novel food and feed products at hand has been conducted on actual customer groups in South East Asia. Our study aimed at better understanding main demand drivers, identify effective value propositions and validate product positioning for a variety of both food and feed insect products in South East Asia. During the study a variety of downstream products derived from insects (*Hermetia illucens, Tenebrio molitor* and *Zophobas morio*) for food, pet food and commercial feed applications were developed and produced in trial quantities. Hypotheses on target audiences were defined, potential value propositions researched and qualitative tests were conducted with members from the target audiences. The results demonstrated a strong awareness of target audiences in the feed sector for commonly promoted value propositions for insect-derived products, particularly economic and health-promoting propositions. For food products, the results revealed a clear disconnect between common reference to traditional regional insect consumption and actual consumer perceptions among target audiences, but an openness in particular target groups for selected products with primarily health-promoting messaging.

# Using outreach to enhance public familiarity and comfortability with entomophagy: let taste do the talking!

Session 11 Consumer attitudes 1

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Most people in USA are reluctant to consider consumption of insects! They find the idea disgusting, repugnant, and illadvised. Their opinion is not derived generally from experiences and exposures to entomophagy, but rather from perceptions of insects ('filthy' creatures) as food. How can the public have more experiences and exposures to entomophagy to shift negative perceptions to more positive ones? One tool to enhance its familiarity and comfortability is to integrate and offer outreach activities focused on insects as food. Outreach is used widely to share new technologies and new research findings with the public or to support programs to educate them on various subjects. Outreach activities are highly popular, extremely successful, and generally well attended. Can outreach provide opportunities for people to gain experience and exposure to entomophagy to improve awareness, perception and knowledge to enhance familiarity and comfortability with the goal to breed public acceptance of insects as food? We think so, especially if we let 'taste do the talking'! This paper summarizes outreach activities conducted at universities and colleges in USA. Some activities reach small numbers, while others reach large numbers. Regardless of number of attendees, activities are important to publicize entomophagy and to maintain its concept in the public eye. For example, the 'Buggy Buffet' has been conducted annually at University of Tennessee for 11 years. As an outgrowth of a Freshman Seminar, this outreach activity has exposed ca. 4,000 people directly to insects as food; if each person shares an experience with a conservative estimate of 10 people, then 40,000 people have been reached indirectly! If attendees post images and comments on Facebook, Twitter, etc., then thousands more have been introduced to entomophagy. If television reporters cover outreach activities, then many more people gain familiarity with insects as food. As more activities are conducted, more people will gain knowledge and realize the importance of insects as food, for humans and for animals, and better understand the role this diverse food source plays in sustainability. This paper also provides an overview of public perceptions related to consumption of insects. This overview will be derived from surveys of people who have (and have not) participated in insect-tasting events. Positive participation in outreach activities changes perceptions and attitudes about insects as food. Thus, it is important that we offer outreach opportunities to allow us to share entomophagy. Set a date, invite the public, prepare the food, and let the taste do the talking!

# Disgust sensitivity towards insects and its relationship with demographic and cultural factors

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Sensitivity to disgust is one of the main factors that predisposes a person to accept or reject a novel food and it can vary significantly among people. This work investigated the impact of individual characteristics and attitudes to certain lifestyles and behaviours on the sensitivity to disgust towards insects as food. The modulation effect of gender and age on insect acceptance was also studied. A questionnaire focussed on socio-demographics, cultural experiences, food habits, sensitivity to hygienic factors, sustainability issues, perception of disgust for various elements, opinions on insects and insect consumption was developed and distributed using a link shared via email and social networks. The survey was completed by 827 subjects. Respondents (49% males, 51% females) was divided into three age classes: the young people (18-34 years), the adults (35-54 years) and the elderly (55-74 years). Results showed a significant effect of both gender and age. The effect of gender on disgust sensitivity for insects was significant when considering the young people, with females being more disgusted than males, while no significant differences were observed between males and females for the adults and elderly. Several differences between young people and the other two classes in matters of disgust sensitivity, attitudes and behaviours were noticed. The effect of the hygiene concern on disgust sensitivity has been demonstrated showing that individuals that believed more in the importance of food safety and sanitary conditions were significantly more likely to be strongly disgusted by insects. Similarly, cultural open-mindedness had also an effect on disgust sensitivity showing that subjects who declared to have lived abroad and those who love learning about different cultures from theirs were less disgusted by insects. On the contrary, subjects afraid to experience different cuisines from theirs and people scared by immigration were more disgusted by insects. Moreover, food consumption habits resulted to influence the sensitivity to disgust. In particular, disgust towards insects and insect flour was positively correlated with the reported sensitivity to disgust for crustaceans, sushi and raw meat. An effect of the surrounding environment was also observed, with subjects living in the city resulted more disgusted by insects than those who live in the countryside. In conclusion, this study allowed to understand some of the cultural and individual factors causing differences in sensitivity to disgust towards insects and provided useful information to contribute to hypothesise future strategies in order to overcome this feeling.

## Consumer attitudes in blind insect based food tastings

#### Session 11 Consumer attitudes 3

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Crickets (*Acheta domesticus*) are interesting creatures that are helping increase sustainability while serving as another food source for the growing population. These insects are eaten by 2 billon people in the world and encompasses a large amount of protein, minerals, and vitamins such as omega 3, calcium, B12, and 6 fatty acids. This research focuses on data collection on individual's views on insects as an alternative food source with a pre and post survey administered during a blind taste test. The survey will be distributed to at least one hundred participants for the analysis. Surveys will also be categorized based on an individual's prior knowledge and comfortability with insects; for example, an entomology student's form would be separated from a student that has a different major that might have less of a well-informed basis of insects. After the tasting is completed the participants will be informed of the ingredients in the food they have tasted as well as played an educational video about the growth of insects in the edible industry and their benefits to the growing population. Once this is completed the post -survey will be administered with the ultimate goal of trying to find out if the exposure to insect base foods effect people's acceptance of alternative food sources.

## The role of chefs in sustained consumer acceptance of insect-based foods

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Insects and insect production have become a relevant argument for those who wish to combine sustainability and culinary innovation. A growing amount of studies have been conducted about the use of insects in human diets, and it is becoming more common to obtain different products that contain insects in supermarkets. However, taste and appearance is a critical element of sustained consumer acceptance, in cultures with and without previous history of insect consumption. Based on five years of research-based gastronomic innovation on edible insects from around the world, conducted by the Nordic Food Lab, University of Copenhagen, this presentation looks at the role of gourmet chefs in shaping food innovation and interdisciplinary research by bridging modern and traditional processing techniques, culture, and traditional-know-how across cultures, in Kenya as well as other countries of the world.

## Social perception on entomophagy in Korean and Ethiopian population

Session 11 Consumer attitudes 5

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We assessed the social perception towards eating insects in two countries viz. Korea and Ethiopia. A semi-structured questionnaire survey was conducted and 329 and 203 respondents of different age groups from Korea and Ethiopia (Adama and Assela city) respectively participated in the survey. Data obtained from the survey was analysed by SPSS. Results revealed 88% of Korean respondents were aware of entomophagy fully or partially presumably suggesting a rich tradition of using insect for food and medicinal purpose. In contrast, only 37% Ethiopian respondents were aware of it. However in both cases the knowledge system was independent of gender respectively for Korean and Ethiopian population). Males were found more inclined to accept insect as food while females were found neophobic for both the countries. Although not statistically significant, in Korean population acceptance of insect as food and food ingredients was found higher among older people perhaps because of familiarity with ancestral knowledge. Positive relation was found between age and ancestral knowledge transmission in Korean population. Few Ethiopian respondents informed that eating insects is relatively common in some places like Benishangul Gumuz region but not all parts of the country. Taste or flavour was found the most important determinant factor followed by nutrition and environmental issues in both the cases. However, many Ethiopian respondents mentioned about religious taboo for eating insects and also many of them do not see insect-eating as culturally superior. The outcomes of the study advocate 'awareness' of food safety, environmental issues among the people could be an instrument for wide acceptance of 'entomophagy'.

# Kenyan consumer's preferences, willingness to pay, and potential demand for Consumer attitudes 6 insect-based food products

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We present results from an empirical study investigating Kenyan consumers' preferences and willingness-to-pay (WTP) for insect-based food products, and the factors that influence these. Additionally, we present results on the impact of tasting experience on consumers' WTP for the products. Assessing the demand for such products, we compare market share predictions for products with and without an insect component. We discuss the implications of the results for food security in Kenya.

## First survey on the insect protein consumption and acceptance of insect farm between high school students in Zambia

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Malnutrition and lack of proteins are two major issues in Zambia, and new sources of protein (like insects) could be available for the local communities. Nutripeople, is a no-profit project aimed to develop small insect farm around Monze district in Zambia. This project was conceived to evaluate the insect consumption and possible impact of a new insect farm system on the protein availability in the district of Monze. This research investigated the food habits of students between 12 to 27 years old in high school in Monze city in January 2018. Survey was provided to a pool of 185 students in a single day. The main submitted questions were about age, sex, number of family members, and the consumption of animal protein (poultry, pork, goat, cow, eggs, dry fish and insect) and vegetal protein (legumes) during a week. The obtained data were distributed in three categories; 12-15, 16-19 and +20 years-old, respectively with 75, 93 and 17 members. The sex ratio was 48.1% male and 51.9% female. The average members per family was 7.3 persons. Of the students to respond, 97.8% indicated they regularly consume legumes as main source of protein, and 48.6% consume them more than 5 times per week. 90.3% of the students answered that they usually consume meat during a week. Among them, the 73.2% of consumption was distributed between 1 to 3 time per week. The most consumed animal protein were eggs and dry fish (95.1 and 93.5%, respectively). Approximately, 39.4% of student answered to usually eat insects and 91.8% eat them between 1 and 3 time per week. The common consumed species are thermites (52.7%), mopane caterpillar (43.2%) and grasshopper (2.7%). Approximately, 99.5% of students were interested to visit the local insect farm developed by Nutripeople project. The local community showed a basic interest in insect consumption and there are the basis for a development of new farms in Monze district in Zambia.

# Latvian consumers' attitude to the use of non-traditional source of protein in food

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In Latvia, as with other Europeans countries, traditional protein sources include meat, fish and milk products, as well as eggs, legumes, and others. Eating insects, although they are an excellent source of protein, comes slowly. Although their usage is of great importance considering the expected large increase in animal-based protein. Besides, there are considerable sustainability issues with the production of the common production animals in the Europe, while the environmental impact of edible insects seems to be less. We determined Latvian consumers' views on the acceptance of insects in their diet as common food. Electronic questionnaires using software VisiDati.lv were used to obtain information on the knowledge of non-traditional protein sources; we also assessed the attitudes towards the use of insects as food. Of the 1,243 respondents, 32% were males and 68% females with an average age of 43 years. Results show that most people have heard of edible insects (81%). However, respondents cannot overcome the instinctive aversion to this kind of food. Ninety-two percent of the respondents would not consider eating insect products. Of all interviewed, 49% like the idea of non-traditional protein sources application in food, but only 7% are willing to start using such products on a daily basis; only 12% is willing to replace a part of products with non-traditional protein-rich products. The major reported barriers to edible insect consumption are: little information available about the importance of alternative protein sources in their diet; lack of information about cooking methods; and restricted availability. Latvian people seem conservative and it will need time before they accept new food items, such as edible insects in their diet.

# Italian consumers' attitude towards insects as food: effect of gender, personality traits, and insect visibility

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The new Regulation (EU) 2015/2283 on novel foods opened the possibility to produce and sell (prior authorization) insects as food (IF) and insect-based products or dishes (IBPD) in European countries. However, very scarce information is available regarding Italian consumers' attitude towards insects as foods. This study explored the potential of insects in the Italian food market. An online survey consisted of three parts: (1) socio-demographic questions; (2) measurement of personality related traits (e.g. food neophobia and sensitivity to core-visceral disgust); and (3) questionnaire on attitude towards insects (willingness to eat several IF and IBPD; reasons for including them into the diet). The survey was completed by 400 Italian subjects (males 46%; 18-75 years). Results showed a significant gender effect, females being more sensitive to disgust than males, less prone to try IF and IBPD and a lower willingness to include insects into the diet. Males would include IF and IBP in their diet because they value insect taste and sustainability properties more than females. A significant negative correlation between the willingness to eat IF and IBPD and both the food neophobia and sensitivity to disgust was observed. It was also noticed that the sensitivity to disgust and the rejection to consume IF and IBP increased as the education level increased. The willingness to eat IF varied as a function of the considered insect species: crickets obtained the highest average score, followed by grasshoppers, bees, mealworms and silkworms, while scorpions and giant waterbugs received the lowest score. Higher willingness was obtained for some insect species (e.g. cricket and silkworm) when considered as ingredient of a IBPD, such as cricket flour pasta and silkworm fried rice, probably because the insects were less visible. We also assessed the effect of three different levels of insect visibility on consumers' acceptability. A liking test was conducted with 58 subjects (males 64%; 19-67 years) on three cricket samples: (1) whole toasted dried cricket (completely visible); (2) focaccia bread with small pieces of toasted dried crickets (partially visible); and (3) cricket flour crackers (invisible). Crackers were significantly more liked than focaccia, while the latter was more preferred than the whole cricket. It showed the strong effect of insect visibility on consumers' acceptability. In conclusion, this study contributed to understand the complexities underlying the consumer attitude towards insects as food and providing new insights that could be taken into account for develop future insects based food products.

## Acceptability of crickets among caregivers and children in Cambodia

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Poor complementary feeding contributes to the global burden of malnutrition, and could be improved by adapting locally available nutrient-dense foods for consumption by young children. To test the acceptability of crickets added in powder form to a traditional porridge with steamed rice and finely chopped vegetables. This was compared to similar porridges with either fish or moringa powder. One hundred and eight healthy children age 6-23 months were recruited for two days of taste testing. Each day, each child received a 100-g portion of a traditional porridge with either 8 g cricket, 8 g fish or 5 g moringa added in powder form. Children were tested for acceptability versus refusal of the porridges. Caregivers assessed the test foods for organoleptic properties, preference and likelihood to use it at home, using a fivepoint Likert scale. Samples of the powders were analysed for nutrient content. Eighty children completed the study. All test foods were acceptable to children and caregivers. All children consumed some of the porridge, with the mean intake in grams was 52 for cricket, 46 for fish and 49 for moringa. Intake was not significantly different between the two days for any foods, nor among the groups (F=0.259, P=0.772). The foods were rated positively by more than 60% of caregivers, with child preference greater among cricket porridge, and higher (77%) than fish (45%) and moringa (54%). Caregivers preferred cricket porridge (95%) over fish (79%) and moringa (83%). Willingness to use it at home was significantly higher for the cricket (73%) and moringa (75%) porridges compared to fish (47%). Processing local nutritious foods into a powder form was an acceptable way for young children to consume crickets, fish and moringa. Three locally available but underutilized foods were processed for consumption by young children in rural Cambodia. Micronutrient content varies widely among insects and fish and among moringa trees grown in different soil and climatic conditions. Recipes which combine local nutritious foods will be better able to contribute to improved nutrient intake within the small portions consumed by young children. In addition to the benefits of utilizing accessible and sustainable foods sources, local foods commonly consumed by adults are acceptable for complementary feeding if processed in a form suitable for young children. These findings point to the potential to leverage local foods, specifically powdered crickets, fish and moringa to improve the diets of young Cambodian children.

#### Insects for food and feed - four challenges

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In general, talk about animals often refers to mammals, sometimes including aquatic species, but only rarely are insects included. This is equally true with regard to research in animal science and animal welfare. This might well mirror the fact that most people mainly consider insects as pests (if meeting many at once) or an 'annoying entity' (if meeting single ones), seldom considering the welfare of the individual animal. Thus, even though there might be fascination with regard to their skills and social capacities (e.g. bees, termites) or their beauty (e.g. bugs and dragonflies in fantastic colours) concern for their individual welfare or moral status is not on the agenda of most people. Hence, any discussion on respect for insect welfare might be perceived as a challenge. This general mind-set is typically not based on empirical evidence regarding insect welfare, but merely reflects old traditions. From a legal and ethical perspective it is however reasonable to expect animal treatment to rest on results of scientific scrutiny. Recent research on some insect species (bees, spiders, crickets) show capacities to adopt to or learn from a situation, some of which are related to pain and/or stress. Since just a few out of the more than one million species of insects have been studied, much research remains and caution of general conclusions concerning the welfare capacity of insects is warranted. Hence, scientific challenges lie already in both mapping capacities and in ethical reasoning on relevance of welfare. Absence of proof should not be considered proof of absence. These challenges only become more pressing as they are linked to two other and interrelated challenges: global food security and climate change. Suggestions that an increase in insect production could ensure a stable protein supply with a low environmental and climate impact coupled with new biotechnological options increasing the usefulness of insects call for ethical reflection on insect welfare and ethics in relation to both mass production and genetic modification/editing. Is genetic modifying or gene editing through e.g. CRISPR-Cas9 less or more ethically challenging when used on insects than when used on mammals or fish? Should production systems take the welfare of individual insects into account? Does the death of 10,000 insects matter more or less than the death of one cow? The paper will elaborate on these and related questions to show the wide range of ethical issues that the development within insects for food and feed raises.

#### Food safety management system in insect farms for human consumption

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Micronutris is the first company in the sector to have implemented a food safety management system based on the requirements of the international standard ISO 22000. At a time when edible insects are rapidly becoming a new emerging market in Europe, the EFSA pointed out in October 2015 that edible insects farms would 'achieve a specific risk assessment taking into account the entire production chain to the consumer'. In December 2015, Micronutris became the first insect farm in the world to obtain the ISO 22000 certification, the international recognized standard for food safety management system. In order to emerge in the insect sector in western countries, one of the main challenges is to provide edible insects that respond to western high quality food production standard. On December 15, 2015, Micronutris became the first insect breeding farm in the world to be ISO 22000 certified, an internationally recognized standard for food safety management. For the sector to emerge in Western countries, one of the major challenges is to provide insects that meet the health requirements in force. The objective of this presentation is to discuss the aspects of agri-food standardization applicable to an emerging sector, to go through the safety points to be taken into account and to approach the integration of an international standard with a large-scale insect production system.

## Edible insects of Korea: policies and progress

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The nutritional potential of edible insects is no longer in doubt. However, the most pragmatic issue remains about the awareness and acceptance of the unfamiliar or little-known food resource. The legislative framework helps to ensure the food safety which is the primary concern of the consumers. Insect-eating is not novel in Korea; the country has a rich history and tradition of using insect as food (silkworm pupae commonly known as 'bundaegi') and medicinal purpose. However, to use it in a broader scale and establish in the industrial domain government has been taking initiative since 2011 and made a list of possible edible insects. Following the preliminary process in 2016 KFDA (Korean Food and Drug Administration) classified cricket (Gryllus bimaculatus) and mealworm (Tenebrio molitor) as normal food. In 2015 the number of insect farms arrived at 724 with a total worth of Korea's insect industry of about 278 million USD which is nearly two times from 2011. Out of the total insect industry, edible insect sector including the medicinal and animal feed section is valued about 143 million USD. With the projection of market growth, the government estimates a market size of about 457 million USD by 2020. Hosting a world symposium on edible insects and showcase traditional dishes including insect cuisine during the recent winter Olympic in PyeongChang lobbying eco-friendly insect protein is a remarkable strategy to encourage the consumer and industry. On the other hand, few incidents of food allergy followed by consumption of insect food have raised concern. Besides, the high pricing of the edible insect is another challenge which should be addressed for inclusion in the routine menu. Furthermore, manipulation of feed on which insects is being reared in order to produce more nutrient-dense insects is another research area yet to be explored from local government, e.g. Gyeongbuk province and central government level.

#### Quality control applications for hyperspectral analysis of black soldier fly insect products

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Cutting edge technologies are a major theme in the developing industry for insects as food and feed. Many logical applications of technologies that are already deployed in food quality control exist. However, the uniqueness of insect products brings opportunities for innovation. One example of this is in the widespread use of spectral analysis for product quality control. Commonly, Near Infrared (NIR) is used for this purpose. However, NIR applications are normally restricted to single point analyses of samples and many systems are tuned for the 750-1000 nm range. In this study, spectra from a hyperspectral camera tuned for the 900-1,700 nm short wave infrared (SWIR) range are used to determine whether relevant information for characterization of insect products exists outside the wavelengths typically included in the NIR spectrum. Furthermore, the hyperspectral system allows area scans of materials, enabling the detection of out of specification elements in the sample. In addition to case studies on classical compositional analyses and quality control based on spectral analyses, perspectives for advanced methods for materials characterization in insect production and the implications for this in food safety are discussed.

## Viability of the liver fluke Dicrocoelium dendriticum in Formica polyctena ants after exposure to different treatments

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Insects for food can either be produced or sampled in the wild. In the temperate parts of the world, ants, like the red wood ant *Formica polyctena*, are foraged locally and appreciated for their interesting smell and flavour. In particular, in foraged insects the presence of food-borne parasites is a concern due to the potential hazards it may cause consumers. The red wood ant *F. polyctena* is an intermediate hosts of the lancet liver fluke *Dicrocoelium dendriticum*, and if infested ants are consumed the lancet liver fluke can infect humans. In this study we developed a viability assay including trypsin treatment of trematode *D. dendriticum* metacercariae from the abdomen of the red wood ant *F. polyctena*. Then we tested the viability of *D. dendriticum* metacecariae in relation to: (1) freezing (-20 and -80 °C); (2) boiling (100 °C); and (3) ethanol (50%). The metacercariae from all treatment groups were recorded as dead. Freezing at -20 or -80 °C for 30 min, boiling for 1 minute or storage in 50% ethanol for 24 hours proved effective in killing the lancet liver fluke *D. dendriticum* metacercariae.

#### Eat insects to save capitalism?

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In this paper I critically discuss the 'entomophagy movement' as a currently developing commodity frontier. The UN-FAO, several state institutions, scientists, so-called entopreneurs and other stakeholders are framing insects as 'food of the future? Due to their high feed conversion ratios, low greenhouse gas emissions and other favourable properties they are said to be an efficient and sustainable alternative to conventional meat, thereby alleviating the ecological crisis, and even a solution to world hunger. I argue that this solution narrative has a technocratic-neoliberal bias and possibly extends rather than solves the problems at hand. Empirical evidence suggests that the growing trade with edible insects reinforces social inequalities and ecological contradictions. Being the causes of world hunger and environmental degradation, both may be seen as rooted in the capitalist world-ecology. From this angle, the appropriation of wildharvested insects and especially the beginning global commodification of farmed insects as (potentially) Cheap Food represent 'innovatively conventional' attempts to keep the current (food) system running. The conventional concept of sustainability largely adopted by the entomophagy movement is defined in narrow quantitative terms and resembles other technofix approaches in not addressing capitalism's deeper 'social' and ecological contradictions. Similarly, insects' promotion as animal feed, their use in biotechnology and for other commercial enterprises – while in principle and to some extent constituting a corrective within the current (ir)rationalities of (food) production – could even create new forms of exclusion and ecological destruction. This 'insect frontier' does not only entail the animals themselves, epitomizing remote 'external' and Cheap Nature, but also respective knowledge, labour, genetic and other extractable resources. All this is supposed to secure prospective accumulation processes while avoiding systemic changes, thereby likely shifting the structural issues of capitalism further into the future. More practically speaking, this suggests awareness of structural issues rather than merely focusing on technical feasibility – as long as insect's potential as an 'alternative' is to be taken seriously in more than economic terms.

## Entomolization: the influence on earth and space before 2030

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The existing number of described insect species is enormous (e.g. 1,000,000 so far), with many more waiting to be discovered. The value and usage of different insect species may give change to the world in terms of their use as food, feed, medicine, and renewable energy. We should promote and explore the possibilities of its technological advancement in mass production development. This presentation will discuss 1.How 'entomolization' as a close-loop key between agricultural supply-side reform and modernization of agricultural technology, which may affect economic, social, and sustainable development. 2. Implementation of insect rearing modules with aerospace-agricultural system Insect production in space could be the solution of high grade protein supply to astronaut, organic fertilizer supply to the hydroponic system for vegetable growth, new materials for basic construction materials which reduce the cost of delivery and loading of the spacecraft.

# Strategy for using insects as alternative protein sources for animal feed and prospects for future production in Poland

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In Poland, high-protein feed resources are in short supply, which contributes to increased soybean meal imports. The growing demand for feed ingredients high in protein and energy has prompted a search for alternative local protein sources. Apart from increasing the area under grain legumes and local soybean varieties, one of such alternatives could be insect protein. There is no tradition of eating insects in Poland, therefore insect breeding programs or processing technologies have never been implemented. Nevertheless, it appears that new technologies for processing insect protein into animal feed could be developed in the nearest future. A research project financed by the National Center for Research and Development, aimed at developing a strategy for insect protein production, will be launched in Poland in 2018-2020. Insect species considered most suitable for large-scale farming under the local climate and environmental conditions will be selected. The principles of insect breeding and farming will be established taking into account quality control and assurance (HACCP). Laboratory analyses of protein extracted from selected insect species will be performed. Feeding trials involving poultry will be conducted on the laboratory and industrial scale. Social surveys on attitudes towards replacing genetically modified protein with insect protein in animal feed will be performed with the use of innovative modelling methods. Various groups of food consumers will be surveyed. In the last stage of the project, a strategy for industrial-scale insect farming and insect protein utilization in animal nutrition will be developed. Commercial insect breeding can be combined with environmental protection because food-processing wastes and by-products can be effectively managed and utilized in all stages of the production process. The strategy will also promote new industrial and business activities among both individual farmers and animal feed mills, thus creating new job opportunities. In the next few years, the implementation of the novel technology for processing insect protein may exert a beneficial influence on the feed protein balance in Poland, and reduce soybean meal imports.

## Possible anti-stress effect of insect foods

Poster 2

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Food generally provides nutrients, sense of tastes and also some physiologically specific functions. Whilst our scientific knowledge of insect as a food has begun to accumulate, there are few reports regarding physiological functionality of insect foods. The Japanese rice grasshopper (Oxya yezoensis) traditionally eaten in Japan and the European house cricket (Acheta domestica) eaten worldwide were used to investigate their possible anti-stress effects. Eighteen 3-weeks old male ICR mice were equally allocated into three groups. All the groups were provided with AIN-93G as basal diet. The control group, cricket group and grasshopper group were supplemented with casein, dried and ground European house cricket and similarly processed Japanese rice grasshopper as protein sources, respectively. The mice fed the designated diets *ad libitum* for 17 days and feed consumption and body weight were recorded. On the 15<sup>th</sup> day the mice were subjected to the 'elevated plus maze test' then 'open field test' on the 17<sup>th</sup> day both of which are commonly used for testing efficacy of anti-anxiety drugs. The behaviour of each mouse was videotaped for five minutes during the tests. In the elevated plus maze test, duration of each mouse remaining on the open arm was measured. Similarly, duration of staying in the central zone was measured in the open field test. The longer the duration of remaining in these areas was interpreted as the less the anxiety felt by the mouse. The mice in both the cricket group and the grasshopper group remained significantly longer on the open arm compared with the control group in the maze test. Whilst this might suggest possible anti-stress effect of the insect diets, there was no significant difference in the open field test. A possible reason for the differences observed in the maze test is that the proteins contained in the insects were degraded during the digestion and absorption processes and converted into various peptides and some of which might have such a physiological property. Since mental stress alleviating effects of the peptides derived from a milk component and eggs have been reported and considering the protein-rich property of insects some peptides having similar effect can also be expected in insect food. Further investigation is needed to look into the mechanism of anti-stress effect and also other functionality of insect foods.

# Improving termite collection for poultry feed in smallholder farms in northern Burkina Faso

# \${protocol}://www.wageningenacademic.com/doi/pdf/10.3920/JIFF2018.S1 - Wednesday, May 30, 2018 1:34:27 AM - Universita' Statale di Milano IP Address:159.149.162.67

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In West Africa, termites are the main source of protein feed in traditional poultry farming. They are used especially to feed chicks and keets. Chippings from termite mounds are collected in the bush. They are not always easily available and collectors often have to travel long distances to collect them. Therefore, more sustainable systems are used by farmers to trap termites on farm, for example through the use of inverted baskets filled with humidified waste or crop residues and placed on small termite nests. The two main termite genera collected using this method are *Macrotermes* and *Odontotermes*. In the IFWA project, the efficiency of these systems is presently being assessed and methods to improve yields are being tested in several regions. In Northern Burkina Faso a comparison of different organic wastes used to attract termites in pots overnight showed very high differences in efficiency between wastes. To collect *Macrotermes* spp., sorghum stem was the most productive attractant whereas, for *Odontotermes* spp., mixtures with cow dung were more efficient. For the same quantity of attractants, clay pots were much more efficient than cans or plastic containers. High variations were observed among seasons, the highest yields being observed during the rainy season, for the two species. In the rainy season, in one night, more than 20 g of dry *Macrotermes* spp. were obtained with 600 g of sorghum stems, on average. There is a strong potential for disseminating the best trapping techniques to replace destructive collections of termite mounds, in particular in regions where the trapping techniques are not commonly practiced and more efficient methods can be found and promoted compared to the presently used ones.

#### Field evaluation of pet food using enzyme hydrolysate of insects

Poster 4

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In order to develop a formulation of functional pet food with anti-allergic effect, insect hydrolysates were evaluated for its properties as a feed additive. Among 88 of insects extracts, extract of *Hermetia illucens* larva which possessed inhibitory effect of interleukine-2 (IL-2) production was selected for additives in the pet food. The extract was made through enzymatically hydrolysis of the larva by trypsin during six hours and then was each added in feeds at a different ratio (0, 1, 2, 3%). From chromaticity of fees, L value(brightness) has shown the tendency of decrease by increasing adding ratio of insect extract. To evaluate properties of insect hydrolysates as pet feed additive, feeding tests of pet foods were evaluated using thirty dogs. More than 93% of the tested dogs preferred pet foods containing less than 1% of insect extract. As a result of purchase intention investigation, pet owners want to buy feeds containing less than 1% of insect extract to feeds containing more than 2% of insect extract. From now on, we will try to register this insect hydrolysate to animal feed additives.

# A comparison of dog reactions to insects and commercial feed aromas – a preliminary study

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The aim of the present study was to investigate the olfactory attractiveness of air-dried insects to dogs. The trial consisted of 35 adult dogs (20 males; 15 females) of various breeds and which were kept as companion animals. The dogs had free olfactory access to selected unprocessed dried insects: mealworm (*Tenebrio molitor*), Turkestan cockroach (*Blatta (Shelfordella) lateralis*), black soldier fly (*Hermetia illucens*), and tropical house cricket (*Gryllodes sigillatus*). Commercial dried and pelleted dog feed was used as a control treatment. Samples (100 g) were located separately in not transparent closed boxes with five perforations in the cover to improve the intensity of the aromas without direct contact with the tested samples. The box was recorded as chosen when the dog showed interest in it for more than 15 seconds continuously per each attempt (3 attempts per dog). The presented study shows that the selected insect species were chosen as frequently as the control group (P=0.03). However, in terms of preferences by dog gender, *T. molitor* was favoured more often by males than by females, which preferred *B. lateralis*. The current preliminary data suggest that the olfactory features of the selected insect species may be attractive to dogs.

# Effect of defatted black soldier fly larvae meal on growth and serum biochemical parameters of *Pelteobagrus fulvidraco*

Poster 6

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A 56-d feeding trial with 480 yellow catfish (Pelteobagrus fulvidraco) juveniles (1.67±0.01 g initial body weight) was carried out to estimate the effect of replacing fish meal with defatted black soldier fly (Hermetia illucens) larvae meal (DBSFLM) on growth performance, body composition and serum biochemical parameters of *P. fulvidraco*. Fish were randomly allocated into four isolipidic (8.91±0.28%) and isoprotein (41.68±0.50%) dietary treatments which were formulated by replacing 0 (T0), 20 (T20), 40 (T40) and 60% (T60) of fish meal (FM) protein with DBSFLM. Each treatment was randomly assigned to triplicate groups of 40 fish per aquarium. Fish were fed twice daily to apparent satiation. With increasing content of DBSFLM, the final body weight (FBW), weight gain rate (WGR) and specific growth rate (SGR) of yellow catfish was increased at first and then decreased. Growth performance of FBW, WGR and SGR in T20 were highest (P<0.05) and higher (P<0.05) than T60, whereas the feed conversion rate was the lowest (P<0.05). The feed intake in T20 and T60 was higher (P<0.05) than that in control and T40 groups. With the increasing content of DBSFLM, the intraperitoneal fat index (IFI) was declining with T60 group was lower (P<0.05) than control. There was no difference of condition factor, viscera index, hepatosomatic index, intestine index and gastric index among treatments. Moisture, crude protein, lipid and ash in whole body were not affected by dietary treatments. Compared with control, the serum cholesterol and triglyceride were reduced (P<0.05) and the high density/low density lipoprotein cholesterol were increased (P<0.05) in T20, T40 and T60 groups. Results suggested that the growth performance and body composition of yellow catfish juveniles was not affected by dietary DBSFLM and the optimal alternative ratio was 20%. The replacement of fish meal with DBSFLM may affect fat metabolism by reducing IFI, serum triglyceride and cholesterol.

# Effect of insect meals in microbiota composition of the gastrointestinal tract of Siberian sturgeon (*Acipenser baerii*)

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Insect meals contain antimicrobial peptides and chitin that can cause a modification in the microbiota of the gastrointestinal tract. insect meals may play a crucial role not only in the absorption of nutrients but also in the gastrointestinal tract immunity, avoiding the colonisation of pathogenic microorganisms in the gastrointestinal tract. The objective of the trial was to study the abundance of five main microorganisms in Siberian sturgeon fed with two type of insect meals. The Siberian sturgeons were fed during 60 days, with three type of diets: (1) 15% *Hermetia illucens* larva-meal; (2) 15% *Tenebrio molitor* larva-meal; and (3) a control diet with only fishmeal. The fish were euthanised. The samples of the gastrointestinal tract content were frozen and prepared according to a standard method to analyse the microbial community by fluorescent *in situ* hybridisation. Four types of oligonucleotide probes were used and the samples were observed using a Carl Zeiss Microscope Axio Imager M2. The number of detected bacteria were expressed in colony-forming units/g of digesta. The analysis performed showed that fish fed with the *H. illucens* diet obtained significant differences in the total number of bacteria from the Enterobacteriaceae family and the *Clostridium leptum* subgroup (P<0.001). The samples of fish fed with *H. illucens* and T. *molitor* diets achieved higher values in *Clostridium coccoides* – *Eubacterium rectale* cluster than the control diet (P<0.001). There were no differences in the content of *Lactobacillus* spp./*Enterococcus* spp. among treatments (P>0.05). The inclusion of *H. illucens* larva-meal contributes to increasing the number of bacteria in comparison to *T. molitor* larva-meal and fishmeal.

## Effect of full-fat insect meals on rainbow trout microbiota

Poster 8

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In aquaculture nutrition, plant meals play an important role. They have partially or totally replaced fishmeal, but some detrimental effect on the intestinal microbiota in carnivorous fish have been observed. Insect meals have appeared as promising protein sources. However, it is not known how these alternative meals affect the microbiota composition. The aim of this trial was to determinate the abundance of certain microbiota in the gastrointestinal tract of rainbow trout fed with four full-fat insect meals. The fish were fed with diets containing 20% of insect meals: Hermetia illucens, Tenebrio molitor, Gryllodes sigillatus, Blatta (Shelfordella) lateralis. The control diet only contained fishmeal. At the end of 60 days, the fish were euthanatized. The samples of gastrointestinal tract content were collected and prepared according to the standard protocols for microbiota analysis, using the microbial community analysis by fluorescent in situ hybridisation. Four types of oligonucleotide probes were used and the samples were observed using a Carl Zeiss Microscope Axio Imager M2. The number of detected bacteria were expressed in colony-forming units/g of digesta. The microbiota analysis shows that fish fed *T. molitor* diet presented the highest content in a total number of bacteria. Besides, fish fed T. molitor and B. lateralis diets presented the highest values in Enterobacteriaceae family as well as *Clostridium coccoides/Eubacterium rectale* cluster (P<0.01). Fish fed *G. sigillatus* diet presented the highest content in the C. leptum subgroup (P<0.001). Although the fish fed T. molitor diet exhibited the lowest value in Lactobacillus spp./*Enterococcus* spp.; fish fed with the control diet presented the highest values (P < 0.001). The quantification of the microbiota shows that fish fed T. molitor and G. sigillatus diet was more affected by the amount and type of bacteria present in the gastrointestinal tract than the control.

## Hydrolysed full-fat insect meals in sea trout nutrition

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*Tenebrio molitor* and *Zophobas morio* both are insects belonging to the Tenebrionidae family of the Coleoptera order. Due to the high-quality protein composition of the larvae, they have shown an immense potential as a fishmeal substitute in aquatic diets. Sea trout (*Salmo trutta trutta*) is one of the promising fish species to be introduced into aquaculture as it has an advantage to tolerate higher summer temperatures. This is important in the long term with regard global warming. The aim of this study was to evaluate the impact of hydrolysed *T. molitor* and *Z. morio* larvae meal as a partial replacement of fishmeal on growth performance and feed utilization of sea trout. This phase lasted 28 days, and at the beginning of the experiment 225 fingerlings of  $5.81\pm0.13$  g were randomly allocated to nine tanks of 60 l of capacity. They were fed with three diets: (1) the control diet with only fishmeal; (2) 10% *T. molitor* hydrolysed meal; and (3) 10% *Z. morio* hydrolysed meal. The animals were farmed under controlled conditions. The average temperature during this phase was  $14.7\pm0.6$  °C and the dissolvent oxygen level was  $7.5\pm0.3$  mg/l. All animals were daily fed with a feed ratio from 3.0 to 2.2% according to body weight and water temperature. At the end of this period, no significant differences among treatments were observed in the growth performance as well as in the feed efficiency (*P*>0.05). Preliminary results show that hydrolysed meal of *Z. morio* and *T. molitor* larvae can be an alternative for partial replacement of fishmeal in sea trout nutrition.

## Growth performance of Siberian sturgeon (Acipenser baerii) fed with insect meals

Poster 10

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The use of insect meals in Siberian sturgeon (*Acipenser baerii*) is a possibility, especially at the fingerling and juvenile phases. Alternative meals are needed that that can replace fishmeal, a natural source of protein for this species. The aim of this trial was to determinate the effect of two insect meals on growth performance. The trial lasted 60 days and in the beginning 180 fish ( $640\pm3.9$  g) were distributed over nine fibreglass tanks ( $600 \text{ dm}^3$  capacity). The animals were kept under natural conditions in summer time. The average temperature was  $19.8\pm1.4$  °C and the average dissolved oxygen level was  $3.8\pm0.9 \text{ mg O}_2$ /dm. The animals were fed with three experimental diets: the control diet (only fishmeal); the *Tenebrio molitor* diet with 15% larva-meal inclusion; the *Hermetia illucens* diet with 15% larva-meal inclusion. All the diets were isonitrogenous (53.20%) and isoenergetic (19.60 Mj/kg). The experiment lasted 60 days, during that time, all the animals were fed every day. The amount of feed ranged from 1.4 to 1.8%, according to body weight and water temperature. During the experimental period, there were also no differences in growth performance as well as in feed efficiency parameters (P>0.05). At the end of the trial, there were also no differences in growth performance as well as in feed efficiency parameters and survival (P>0.05). This trial shows that the inclusion of 15% of *H. illucens* and *T. molitor* meals in the diet of Siberian sturgeon is a valuable alternative to replace fishmeal during the juvenile phase.

## Full-fat insect meal in rainbow trout nutrition

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The use of full-fat insect meals in fish nutrition is a natural way to provide protein of high quality and can partially replace fishmeal in farmed fish species. Moreover, rainbow trout in nature eat insects, especially aquatic insects at the first stage of their life. For that reason, the aim of this trial was to use five full-fat insect meals in the nutrition of rainbow trout and to evaluate how these alternative sources affect growth performance. The trial lasted 60 days, and 1,950 fingerlings  $(53.39\pm3.74 \text{ g})$  were distributed into 15 tanks (600 dm<sup>3</sup> capacity). The fish were kept under natural conditions during summer time. The temperature ranged from 7.1 to 20.6 °C and the oxygen level from 4.3 to 11.2 mg  $O_2$ /dm. There were five diets, four of them with 20% of full-fat insect meals. The diets were isonitrogenous (45.03%), and isolipidic 20%. Each diet included one type of insect meal (Blatta (Shelfordella) lateralis, Gryllodes sigillatus, Tenebrio molitor and Hermetia illucens); the control diet was based on fishmeal. The animals were fed every day and the daily ratio ranged from 1.0 to 2.5% of fish body weight, also depending on temperature. The fish were weighed periodically to evaluate growth performance and feed efficiency. During the experimental period, there were no significant differences among treatments (*P*>0.05). At the end of the trial, fish fed *B. lateralis* and *T. molitor* achieved the best growth performance, and the fish fed *H. illucens* diet obtained a growth performance similar to fishmeal diet. The lowest growth rate was obtained with G. sigillatus diet (P<0.05). In feed efficiency, fish fed the G. sigillatus diet exhibited significant differences in comparison to the rest of treatments (P<0.05). We concluded that it is possible to include 20% of B. lateralis, T. molitor and H. illucens full-fat meals to achieve better growth performance in rainbow trout nutrition.

# Black soldier fly (*Hermetia illucens*) larvae – a superior protein source in aquaculture feeding

Poster 12

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Demand for protein is expected to grow significantly as an increasingly affluent global population reaches over 9 billion by 2050. At the same time, supply will be constrained in the face of scarce resources, land and water. These trends illustrate the need for an increase in the production of alternative sustainable protein sources. The high feed conversion efficiency of the black soldier fly (Hermetia illucens) compared with conventional livestock, its high nutritional value and reduced environmental impact, makes BSF an ideal sustainable protein source. Dried black soldier fly larvae (BSFL) contains 40% protein and 35% fat. Extraction of the larvae protein produce a meal with high nutritional value (>55% protein), allowing for formulation of balanced diets in a wide range of feed applications. Our studies aim to evaluate the potential of BSFL as a nutritional feedstuff in aquaculture diets. A study with prawns fed diet containing frozen BSFL. Macrobrachium rosenbergii prawns at post larval stage were fed with frozen BSFL for a total duration of 51 days in three replicates of 20 animals in each replicate. Frozen BSFL was obtained from BioBee-Biological Systems Ltd. There was a 54.9% increase in average daily gain (ADG) and 32% decrease in feed conversion ratio (FCR) of M. rosenbergii postlarval prawns with the inclusion of frozen BSFL in the diet. A study of total substitution of fishmeal protein source with BSFL protein meal in rainbow trout diet. A total of 720 rainbow trout of  $11.4\pm0.2$  g) were randomly allotted to three experimental diets containing overall 44% crude protein and 21% crude fat. Rainbow trout were fed for a total duration of 56 days. The data were analysed using JMP 11 software. The Tukey-Kramer method was used to adjust for pairwise comparisons and to perform mean separations. For all analyses, significance was declared at  $P \le 0.05$ . The initial body weight, growth responses and carcass compositions observed from the experiment were similar for all replicates. For SGR and FCR the differences between the experimental groups were insignificant. The trial results suggest a potential for BSFL to be incorporated into rainbow trout and M. rosenbergii diets, thereby reducing the reliance on fish meal, and making BSFL a promising alternative protein and fat source for aquaculture feed.

# Effect of *Tenebrio molitor* oil on growth performance and nutrients digestibility in broiler chickens

Poster 14

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The aim of the study was to investigate the effect of total replacement of palm oil and poultry fat with Tenebrio molitor oil in broiler chicken diet on the growth performance and gastrointestinal tract pH and nutrient digestibility. A total of 72 seven-day-old female Ross 308 were used in this experiment. At arrival, the birds were weighted and randomly distributed to three different groups, 12 replicates per group and two birds per replicate. The birds were kept for 30 days in metabolic cages. The birds had free accesses to feed and water during the whole period of the experiment. The basal diet was formulated on maize and soybean meal basis. Five percent palm oil, poultry fat or T. molitor oil was added to the diet depending on the treatment. No additives were added to the diet and 0.3% of TiO<sub>2</sub> was added as an internal marker for digestibility calculations. The measurement of the body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) was conducted during day 7, 14, 21 and 30. On day 30, all birds were sacrificed and samples for apparent ileal digestibility were collected as well as the pH of crop, jejunum, and caeca were measured. Data were tested using the GLM procedure of SAS software. In the experiment, means were separated using Duncans' tests following one-way ANOVA. In all periods of the experiments (7-14, 14-21, 22-30 or 7-30), the application of T. molitor oil to the basal diet did not have any effect on the BWG, FI or FCR in comparison to the treatments with poultry fat or palm oil. The pH value of the crop, jejunum and ceacal digesta did not differ significantly among all treatments (P=0.25, P=0.98, P=0.72, respectively). The value of apparent ileal digestibility of crude protein, ether extract, and apparent metabolizable energy did not show any differences among all treatments (P=0.31, P=0.36, P=0.24, respectively). The use of T. molitor oil in broiler chicken diet did not show any effect on the growth performance neither the ileal nutrient digestibility in comparison to poultry fat or palm oil. According to our study, we conclude that the *T. molitor* oil can be applied in broiler chicken nutrition without any adverse effects on the performance.

# Effect of *Tenebrio molitor* oil on selected blood parameters and internal organs weight of broiler chickens

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The aim of the present study was to evaluate the effect of total replacement of palm oil and poultry fat with Tenebrio molitor oil in broiler chicken diet on different blood parameters and internal organs weight. A total of 72 seven-day-old female Ross 308 were used in this experiment. At arrival, the birds were weighted and randomly distributed to three different groups, 12 replicates per group and two birds per replicate. The birds were kept for 30 days in metabolic cages. The birds had free accesses to feed and water during the whole period of the experiment. The basal diet was formulated on maize and soybean meal basis. Five percent of palm oil, poultry fat or Tenebrio molitor oil was added to the diet depending on the treatment. No additives were added to the diet. At day 30, ten birds per replication per group were randomly selected and blood samples were collected from the wing vein. Serum was obtained by centrifugation (Micro 220R, Hettich, Tuttlingen, Germany) at 1000×g at 8 °C for 10 minutes and stored at -20 °C until analysis. The following blood levels were determined: non-esterified fatty acid (NEFA), glucose, triglycerides (TG), cholesterol, total protein, and albumin. The weight of liver and pancreas was measured and the organs weights in relation to body weight (BW) (% of BW) were calculated. Data were tested using the General Linear Models (GLM) procedure of SAS software. In the experiment, means were separated using Duncans' tests following one-way ANOVA. No statically significant differences were observed on the NEFA, glucose, total protein, and cholesterol. However, the use of *T. molitor* oil significantly decreased the level of triglyceride in comparison to poultry fat and palm oil (P=0.04). Statistical differences were observed on the liver (*P*=0.03) weight, where the lowest value was observed in *T. molitor* oil treatment. While no statistically differences were observed on the pancreas involvement in the body weight. The use of T. molitor oil in broiler chicken diet reduced the level of triglyceride in blood serum as well as the liver weight in relation to the live body weight.

# Total replacement of soybean oil with *Tenebrio molitor* oil affects the fatty acid profile of breast meat in broilers

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The aim of the study was to investigate how oil obtained via super-critical CO<sub>2</sub> extraction from *Tenebrio molitor* (TM) and Zophobas morio (ZM) larvae affects the lipid fatty acid composition of breast muscle tissue of broiler chickens. A total of 108 one-day-old female Ross 308 chicks were used in this experiment. Birds were fed soybean-maize diets developed by replacing 50 g/kg of the basal diet with soybean oil (SO), TM and ZM. The birds were randomly assigned to three dietary treatments. Each treatment had 12 replicates and three birds per replicate. Birds were kept in metabolic cages over a 28 days period. After slaughtering the breast muscle was collected for further analyses. Lipids from breast tissue were extracted using the procedure described by Głogowski et al. and fatty acid methyl esters were determined by gas chromatography according to Cieślak et al. Data were tested for normal distributions using the Kolmogorow-Smirnov test. An analysis of variance was conducted using Bartlett's test. The significance of differences among groups was determined with Duncan's multiple range tests at the significance level of P < 0.05. The fatty acid composition of breast muscle shows significant differences in the values of C18:3 c9c12c15 (P<0.001), C20:4 (P=0.01), and C22:6 (P=0.001) in both insect oil supplementation groups. TM oil addition resulted in the lowest concentration of 16:0 fatty acid (P<0.001) and the highest of C18:3 n-6 (P=0.024). ZM oil lowered values of C18:2 c9c12 (P=0.021), C22:0 (P=0.036), as well as C24:1 (P=0.015). Supplementation of insect oils had an effect on the fatty acid profile of breast tissue by increasing the value of C18:1 c9 (P<0.001). However, only the ZM oil group was characterized by the highest values of C16:0 (P<0.001), C16:1 (P=0.004), and C23:0 (P=0.001). The addition of both insect oils increased MUFA (P<0.001) and total C18:1 (P<0.001) in the breast muscle. The results of the current study suggest that inclusion of TM, obtained by using super-critical CO<sub>2</sub> extraction, and added to the basal diet of broiler chicken had a positive effect on the breast meat fatty acid content, which is a component of consumer quality requirements.

# Effect of total replacing soybean oil with *Tenebrio molitor* oil on growth performance of *Poster 16* broiler chickens

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The aim of this study was to investigate how oil obtained via super-critical CO<sub>2</sub> extraction from *Tenebrio molitor* (TM) and Zophobas morio (ZM) larvae affect the growth performance of broiler chickens. Two independent experiments were conducted on 72 and 108 one-day-old female Ross 308 chicks. Birds were fed soybean-maize diets developed by replacing 50 g/kg of the basal diet with various fats, such as soybean oil (SO) and TM (Exp. 1) or SO, TM and ZM (Exp. 2). In both experiments, each treatment had 12 replicates and three birds per replicate. Birds were kept in metabolic cages over a 28 day period. Birds were weighed on days 1, 7, 14, 21, and 28. The body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were calculated for days 1-7, 7-14, 14-21, 21-28, 1-28. Experiment 1. In the first period, no significant differences among the treatments were recorded. A positive effect of TM was observed from days 7 to 14 as BWG (*P*<0.001), FI (*P*<0.001) and FCR (*P*<0.001) increased. From days 14 to 21, a similar influence of insect oil was recorded, except that BWG (P=0.545) did not differ among groups. From days 21 to 28, no changes in growth performance were observed among groups. In the entire experiment, TM usage caused a decrease in FI (P=0.013) and FCR (P=0.034), though BWG was not affected (P=0.991). Experiment 2. The usage of insect oils did not affect the growth performance parameters. Only the 14-21 day 21-28 day periods were different in BWG among treatments. The application of SO and of TM did not differ in any of the above-mentioned periods, but ZM lowered BWG (P=0.041) in comparison to the TM treatment in the period from days 14-21. However, in days 21-28, dietary supplementation of ZM significantly improved BWG compared to the control (SO) treatment. Both TM and ZM increased the FCR value (P=0.048) until day 7. From day 14 to 21, the highest FCR value was observed in the ZM group. The results of the current study suggest that inclusion of TM, as well as ZM, obtained using super-critical CO<sub>2</sub> extraction can be used to completely replace SO in broiler chicken diets without any adverse impact on the growth performance.

# Effect of full-fat insect meal addition in broilers diet on microbiota in the gastrointestinal tract

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The aim of the study was to evaluate the effect of selected full-fat insect meals fed 'on top' to broiler chickens on the gastrointestinal tract microbiota. In the trial, 400 one-day-old, female ROSS 308 chicks were used. Birds were placed into four treatments containing 10 replicates per group and 10 birds per pen. Blatta (Shelfordella) lateralis (SL20), Tenebrio molitor (TM20) and Hermetia illucens (HI20) were used at 0.2%. Populations of microbiota were determined in the crop, ileum and caecal digesta by FISH method according to Józefiak et al. Digesta pH was not altered by the application of different full-fat insect meals; however, even in the crop, statistically significant changes in microbiota were observed. In the TM20 treatment, the lowest counts of the Bacteroides-Prevotella cluster were observed (P=0.001). Compared with SL20 and TM20 treatments, HI20 lowered Clostridium leptum subgroup counts and increased those of the Clostridium coccoides-Eubacterium rectale cluster. Moreover, in the HI20 treatment, the highest numbers of Lactobacillus spp./ *Enterococcus* spp. were observed compared with those of the negative control (NC) and SL20 treatments. In the ileal digesta, the C. coccoides-E. rectale cluster count was the highest among all experimental treatments, but the results were only significantly different compared with the NC results (*P*<0.001). The lowest *Lactobacillus* spp./*Enterococcus* spp. counts were in the HI20 treatment, whereas an increase was observed in the TM20 treatment. In the caecal digesta, counts of Bacteroides-Prevotella, C. coccoides-E. rectale clusters and Streptococcus spp./Lactococcus spp. were the highest in the HI20 group. HI20 addition also resulted in increased counts of Lactobacillus spp./Enterococcus spp. compared with those in the control and TM20 group. We conclude that supplementation with full-fat insect meals in relatively small amounts, i.e. 0.2%, in the diet of broiler chicken can have a positive effect on microbial composition in of the GIT. The study was supported by Bridge Alpha, HiProMine S.A – The National Centre for Research and Development.

# The effect of *Blatta (Shelfordella) lateralis* full-fat meal supplementation in broilers diet <sup>Poster 18</sup> on growth performance

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The aim of the study was to evaluate the effect of *Blatta (Shelfordella) lateralis* full-fat meal fed 'on top' to broiler chickens on their growth performance parameters. In the present study, 500 one-day-old, female ROSS 308 chicks were distributed into five groups with 10 replicate pens of 10 birds each. The experiment lasted for 41 days. *B. lateralis* was added in amounts of 0.05, 0.1 and 0.2%. The growth performance parameters, i.e. body weight gain (BWG), feed intake (FI) as well as feed conversion ratio (FCR) were evaluated. In the periods 10-21 days and 1-21 days, supplementation of 0.2% *B. lateralis* full-fat meal improved BWG. The final BWG (1-41 days) in this treatment improved by approximately 3.5%, although the difference was not statistically significant (P=0.099). The FI also improved in the 0.2% *B. lateralis* treatment in the periods 1-10 days and 1-21 days. The FCR in the 0.2% *B. lateralis* treatment in the period 1-21 days was better than that in the positive control (P=0.045) but did not differ from that in the negative control. Based on the results of the present study, we conclude that supplementation with full-fat insect meal made by *B. lateralis* in relatively small amounts, i.e. from 0.05 to 0.2%, in the diet of broiler chicken diets can have a positive effect on their growth performance. The current study was supported by the following grant: Bridge Alpha, HiProMine S.A -The National Centre for Research and Development.

# Efficiency of converting manure into protein feed and organic fertilizer by Hermetia illucens larvae and bacteria

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Conversion efficiency of chicken manure with *Hermetia illucens* larvae (BSFL) and synergistic bacteria for producing larvae as feed stuff and with decomposing agent for producing organic fertilizer was examined. One million 6-day-old BSFL were inoculated into 1000 kg chicken manure with, or without, *Bacillus subtilis*. Conversion efficiency and larval production were determined after conversion of 13 days. Inoculation of manure with *B. subtilis* increased larval production by 16% and enhanced manure conversion by 13.1%. BSFL weight increased 15.9% when reared in manure inoculated with *B. subtilis*. After eleven days fermentation, the final product produced from conversion residue inoculated decomposing agent was a higher quality (germination index >92%) than the residue from the control (germination index ~86%). Activity patterns of targeted enzymes also indicates greater residue maturity, stability and thus use as an organic fertilizer. Furthermore, through the integration of these methods (BSFL, bacteria inoculation, and fermentation), the conversion process required only 24 days.

# Growth of Hermetia illucens on waste from mass rearing of other insect species

Poster 20

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Recent interest in integrating insects into the human food chain, as a sustainable protein source for both food and animal feed, has led to a growing number of insect farms establishing across Europe. Cricket and grasshopper species are suitable for human food applications and, despite their higher production efficiency than traditional livestock, their farming still produces substantial quantities of organic waste; consisting of frass, exuviae and uneaten food. Here we assessed the potential use of Hermetia illucens (Dipetra: Stratiomyidae), the black soldier fly (BSF), as a bioaccumulator to recapture the nutrients from this waste for potential applications in the animal feed, pet food, biofuel or pharmaceutical industries. BSF larvae were reared on three experimental diets: (1) cricket farm waste; (2) locust farm waste; and (3) the Gainesville diet, used as a control. Farm wastes were used either unmodified or finely chopped giving five experimental conditions. Trials were started with young handling larvae, provided feed *ad libitum* and reared in a climate chamber. The impact of experimental condition on BSF mortality, developmental time, final larval mass, and adult emergence was assessed. Adult flies were successfully reared on all diets, demonstrating their suitability for BSF growth. The final weight of larvae reared on cricket waste was significantly higher than those on the locust and control diet. Chopping the locust waste did not affect larval weight. Diet did not significantly affect time taken to reach the prepupal stage, but chopping the locust waste diet significantly slowed the developmental rate. Mortality rate was low and not significantly affected by diet. We conclude that among substrates suitable for rearing *H. illucens*, the waste from farming locusts and crickets represents another opportunity for this interesting saprophagous species. The bioconversion ability of H. illucens means that wastes from the edible insect industry represent a new feed source for the mass rearing of this species, providing the opportunity to establish a circular nutrient economy.

# Effect of increased prepupae density in pupation crates on the emergence rate of black soldier flies

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When implementing a decentralized urban black soldier fly (BSF) waste conversion system, one objective is to make best use of the space available. For the BSF nursery, as implemented by the FORWARD project in Indonesia, the longest retention time is in the pupation unit where prepupae enter the pupation stage and transform into adult flies. Increasing the density of prepupae in the pupation crates could significantly improve the space footprint of the nursery. In the research presented here, eleven different densities of prepupae in 60×40 cm pupation containers, ranging from 2,500 to 7,500 prepupae, were tested to measure the effect on the emergence rate of the flies. A 'dark cage' was filled with 16 crates containing prepupae one of one density setting. Eight days after and until the moment the dark cage was dismantled, the emerged flies were harvested by connecting a 'love cage' to the dark cage every 24 h. Results show an emergence rate ranging from 71.6 to 92.2% in the eleven dark cages. The mass of the prepupae ranged from 13.43±0.45 to  $15.15\pm0.61$  g for 100 prepupae where the mass of the flies ranged from  $0.76\pm0.01$  to  $0.98\pm0.05$  g for 10 flies. During the 67 days of data collection, the average daily ambient temperature ranged from 26.23±2.47 to 30.12±3.86 °C where the average daily relative humidity ranged from 70.35±14.78 to 92.33±7.64%. Analysis of data shows no relation of density of prepupae in the pupation crates and the emergence rate of the flies. Furthermore, no observable relation was found between the mass of the prepupae or flies in a dark cage and the emergence rate of the flies from that dark cage. The large variation in the mass among the flies might rather be related to the natural weight differences between males and females. Lastly, no relation between the temperature or relative humidity and the emergence rate of the flies could be detected. The large variation in relative humidity was due to the rainy season climate conditions. It can therefore be concluded that these increased densities did not affect emergence and densities could even be increased further, before noticing a drop in emergence. A broader range from 7,500 to 20,000 prepupae per container will be used for the next trials to narrow the tipping point between higher density and reduced emergence.

# Effect of larval density of black soldier fly (Diptera: Stratiomyidae) on bacterial succession in diets and larval gut

Poster 22

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Black soldier fly (*Hermetia illucens*) larvae are efficient bioconverters of many types of organic waste, and they can provide an alternative protein source for animal feed. As consumers of dead organic matter, black soldier fly larvae interact with a complex microbial community of decomposers. A growing body of literature shows that microbial community composition changes in the presence of larvae of a number of fly species. The larval gut could be a major player in this change, as passage of ingested food through the gut will expose the food-borne bacteria to niches of varying pH, aeration, and chemicals (enzymes, antimicrobial peptides). However, the majority of the microbiome studies on black soldier fly larvae have not included controls of substrates without larvae, sampled a single time point or only sampled the larval gut. These limitations make it hard to draw conclusions on the existence of a typical larval gut microbial community and on the role of black soldier fly larvae in affecting the microbial community. In this study, we aim to address these limitations and assess the effect of larval density on bacterial succession in three different diets (chicken feed, chicken manure, and an oilseed residue diet) and the larval gut. We sampled total DNA from both substrates and larvae over time and included controls of diets without larvae. The bacterial 16S rRNA gene (region V5-V6) was sequenced using Illumina HiSeq sequencing. Preliminary results seem to suggest that black soldier fly larvae alter both substrate- and gut bacterial community composition depending on diet and larval density, and that similarity between substrate and larval gut communities is lower in chicken manure than in chicken feed and the oilseed residue diet.

# Evaluation of adults density of *Hermetia illucens* in the production of eggs under controlled conditions

Poster 24

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*Hermetia illucens* (Diptera: Stratiomyidae), also called black soldier fly is one of the most promising waste-eating insects, especially in urban, industrial and agricultural environments. This fly can develop in a variety of fresh or decomposed organic matters. This nutritional flexibility can make them easy to use using low-value food sources, which makes them ideal for large-scale farming. However, efficient egg production is one of the main obstacles associated with the production of larvae of this fly. The purpose of the present work is to determine the density of adults needed to optimize the reproduction and production of black soldier fly eggs. One experiment with four treatments was carried out to explore the density parameter related to mass production of eggs: treatment A (colony box  $40 \times 40 \times 40 \times 40$  cm with 1000 adults), treatment B ( $40 \times 40 \times 40$  cm box colony with 2,000 adults), treatment C ( $40 \times 40 \times 40$  cm box colony with 3,000 adults), and treatment D ( $40 \times 40 \times 40$  cm box colony with 4,000 adults). All these treatments were carried out under artificial and controlled conditions ( $25 \pm 5$  °C,  $60 \pm 10\%$  relative humidity, light:dark 8:12 hrs). It was observed that when the density is around 3,000 adults per cage, and when the space per fly is around 21 dm<sup>3</sup>/fly the number of the eggs are the highest. These results could indicate that the adults of black soldier fly require a certain density in order to perform the mating ritual. Therefore, it is one of the keys to take into account in large-scale production of *H. illucens*.

# Developmental plasticity: larval conditions and adult investment in morphological traits in the black soldier fly

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Furthering our understanding of the biology of the adult black soldier fly, *Hermetia illucens*, is important for ensuring consistent production of this commercially important species. Larvae of this species are frequently reared in high densities and on nutritionally variable diets, yet the impact of these conditions on the adult life stage is poorly understood. In some insect species, the conditions that the larvae experience can influence the morphological traits expressed in the adult, known as developmental plasticity. We investigated whether developmental plasticity is found in the black soldier fly and how diet and density influence the sensory and reproductive morphology of the adult fly. Our preliminary results indicate that larval densities. We also found that sex and body size, rather than larval rearing conditions, predicted wing morphology and eye size.

# Effects of juvenile hormone analogue on the development of black soldier fly larvae and adult emergence

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*Hermetia illucens* L., also known as the black soldier fly, belongs to the fly family, Stratiomyiidae. Black soldier fly (BSF) larvae are capable of transforming livestock manure and kitchen waste, into biomass rich in protein and fat. Resulting larvae can then be used as an animal feed for aquaculture. Juvenile hormone (JH) regulates many biological features of insects including insect development, metamorphosis, and reproduction. In this experiment, the effect of different levels of juvenile hormone analogue (JHA) on *H. illucens* larval development and adult emergence was investigated. JHA was added to the artificial diets and *H. illucens* larvae allowed to feed. In this experiment, 6 days old BSF larvae were fed on diets which were mixed with different concentrations of JHA. The treatments included JHA concentration of 1, 2, 4, 8 and 16 µg/ml. The control group was the larval diet treated with water. Larval development was completed first with the 2 and 4 µg/ml treatments. Average weight increase of per larvae was 0.1839 and 0.1857 g, which was significantly higher than the other treatments. Survival rate of all groups were over 95%; however, survival rate decreased with increasing concentrations of JHA. Material consumption rate of the 4 and 8 µg/ml treatments were 53.87 and 53.43%, which was significantly greater than recorded for other treatments. Adult emergence rate of the two highest treatments were significantly lower than that of other groups at 39.33 and 33.67%, respectively. No significant difference was found among other groups. Time required for adult emergence of the treatment with the highest rate of JHA was significantly longer than that of the control, which inhibited the adult emergence.

# Environmental and substrate effects on the pupation and adult emergence of *Hermetia illucens* (Diptera: Stratiomyidae)

Poster 26

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The black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), is distributed throughout the world. The larvae can feed on animal manure and decaying organic waste. They convert waste during their larval feeding stages. We investigated the optimization of the nonfeeding stages of development. The objective was to determine the effect of different environmental factors and pupation substrates on the time of post-feeding development and pupation and the success of adult emergence. Three pupation substrates were compared: wood shavings, tailing (bran and sub-powder 1:1, mixed with water to feed the larvae in the remaining residue), and nothing. Also three environmental factors were studied: temperature (24, 28 and 32 °C), pupation substrate thickness (1, 5 and 9 cm), and pupation substrate moisture content (10, 20 and 30%). Postfeeding larvae took longer to reach eclosion in the absence of a pupation substrate, while eclosion was reached soonest in tailing. Also, fewer adults emerged when a pupation substrate was not provided. The moisture content of 30% of the tailing substrate had the best effect. Zhiqiang Chai *et al.* found that developmental duration decreases with increasing temperature; *Ptecticus aurifer* Walker prepupa weight decreased with increasing temperature. Solve solve solve solve solve solve solve solve solve are: tailing substrate, and 28-32 °C ambient temperature.

# The effect of feeding substrates on the growth of Hermetia illucens

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The aim of the study was to evaluate the effect of selected food waste (ReFood) on the growth parameters of *Hermetia illucens*. The following substrates were used: T1 – wheat bran, T2 – carrots, T3 – cabbage, T4 – potatoes, and T5 – a mixture of all above-mentioned materials. Each group contains 10 replications with two grams of larvae. The trial lasted 13 days. We determined: the average weight of 100 larvae (AvW), body with gain (BWG), dry matter intake (DMI), feed conversion ratio (FCR), waste reduction rate (WRR), and waste reduction index (WRI). The highest AvW in case of the entire experimental period was noticed in the T5 group (P<0.001). However, improvement of the total weight of larvae was observed in both the T3 and T5 treatment (P<0.001). Simultaneously, the lowest FCR value was observed in these groups (P<0.001) in comparison to other treatments. The current study suggests that the usage of wheat bran (T1), as well as potatoes (T4) as a substrate for *H. illucens* rearing, is not efficient. Furthermore, the most effective medium for rearing larvae is cabbage (T3). The selection of ingredients used as a mixture of food waste is crucial to improving the efficiency of the growth performance of *H. illucens*. This study was performed in the frame of the IN OIL project: an innovative method for bio-conversion of by-products from food processing industry financed by the National Centre for Research and Development within the Lider VII Programme No. LIDER/5/0148/L-7/15/NCBR/2016.

# Effect of feeding substrates on the chemical composition of Hermetia illucens

Poster 28

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The aim of the study was to evaluate the effect of selected food waste (ReFood) used as feed substrate on the chemical composition of *Hermetia illucens*. The following substrates were used: wheat bran, carrots, cabbage, potatoes, and a mixture of all above-mentioned materials. Each group contains 10 replications with two grams of larvae. The trial lasted 13 days. The following analyses were performed on the larvae: dry matter, crude ash, crude protein, and ether extract. The usage of carrots, cabbage, as well as a mixture of all selected ingredients, improved the fat content. However, the highest value of crude fat was noticed in the carrots treatment. In general, the value of crude ash in each experimental groups was at the similar level. Furthermore, the chemical composition of *H. illucens* larvae varied in terms of crude protein content. The highest crude protein level was observed in the wheat bran group (55.23%) contrary to the potatoes group where the lowest (41.45%) value was noticed. The current study suggests that the usage of selected food waste may affect the chemical composition of *H. illucens* larvae. This study was performed in the frame of the IN OIL project: An innovative method for bio-conversion of by-products from food processing industry that was financed by the National Centre for Research and Development within the Lider VII Programme No. LIDER/5/0148/L-7/15/NCBR/2016.

# Growth performance of the black soldier fly (Hermetia illucens) on by-products from brewing production

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Hermetia illucens (Diptera: Stratiomyidae), also known as the black soldier fly, is considered an interesting candidate as alternative source of protein for livestock. Larvae of this species are able to efficiently bio-convert organic waste material into insect biomass. In addition, larvae can consume twice of their weight per day of waste, accumulating high amounts of protein and fat. The choice of the correct rearing substrate is essential in order to contribute to the disposal of waste or by-products obtained from the various stages of the industrial food production, that could hardly find other utilization, and to maximize the production of black soldier fly prepupae. Moreover, it is important to identify a lowcost diet with no competition with animal or human consumption. Among numerous by-products of vegetal origin, in this study black soldier fly larvae were reared on the following substrates originating from the brewing production: brewer's spent grain, trub, and a mix of the two by-products (50 and 50%). The influence of the rearing substrates of the different life-history traits was observed. In particular, we considered the survivorship of the different developmental stages, the larval final weight, the duration of the larval period and the emergence of adults. Larvae could complete their development on the three substrates tested. Nevertheless, some differences were observed on the different parameters. In particular, the mixture of the two by-products resulted in a faster growth of the larvae that took less days to reach prepupal stage than the ones grown on the single by-product. The same trend was noticed on the final larval weight. The mortality of the larvae was significantly higher on those grown on brewer's spent grain, while no differences were noticed among the other substrates. This study showed the possibility to rear the black soldier fly on different byproducts coming from the brewing production industry, that can therefore represent an interesting rearing substrate for the insect. More research is needed to optimize the diet for a possible use in mass rearing system.

# The influence of diet on the morphofunctional properties of *Hermetia illucens* (Diptera: Stratiomyidae) larval midgut

Poster 30

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Hermetia illucens (Diptera: Stratiomyidae) is among the most promising organisms for the bioconversion of organic waste in proteins for feed production, because the larvae are able to grow on a wide variety of organic substrates and the dry-matter of the prepupae contains a very high percentage of protein with high nutritional value. One of the potential substrates for bioconversion by *H. illucens* is Fruit and Vegetable Waste (FVW), which could be provided in large amounts by large-scale retail trade and wholesale markets. However, in view of a possible application of this system, it is fundamental to evaluate the biological performance and the morphological, physiological, and molecular responses of *H. illucens* reared on this food substrate. In the present study we compared larvae reared on a standard diet for dipteran larvae and on FVW. In particular, after evaluating the growth performances, we focused our attention on the midgut, which is responsible for nutrient digestion and absorption. The morphological changes occurring in this organ after the ingestion of the two diets were investigated by optical microscopy. Moreover, an evaluation of midgut functionality was performed. An analysis based on enzymatic histochemistry was carried out to evaluate specific differences in lipids and glycogen accumulation in midgut cells. Finally, we assessed the activity and the mRNA expression levels of enzymes involved in digestion, focusing on proteolytic and amylolytic ones. Our results demonstrate that the extraordinary feeding plasticity of this insect corresponds to an extraordinary biological, morphological, physiological and molecular plasticity, that allows larvae to perform an effective bioconversion of FVW, opening up interesting application perspectives. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550).

# Effect of inorganic salt on Hermetia illucens growth

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The effect of different inorganic salts on the growth and development of the *Hermetia illucens* larvae were studied. To bran and tail powder, distilled water was added with different concentrations of NaHCO<sub>3</sub> and KHCO<sub>3</sub>: 0.1, 0.5 and 1.0 g/ml. The different inorganic salt solutions and the concentrations had an effect on the growth and development of the *H. illucens* larvae: it promoted growth and the accumulation of biomass. The addition of 0.5% NaHCO<sub>3</sub> salt solution had an effect on the prepupa stage of *H. illucens*. These studies can be useful for large-scale rearing of *H. illucens*.

# Towards optimization of slaughtering methods for larvae of the black soldier fly (*Hermetia illucens*)

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The feeding of monogastric animals is largely based on high grade primary cereals and oilseed commodities. However, the pressure imposed by the agrifood industry on these ingredients is becoming increasingly important. Insects are a promising alternative feed considering the high quality of lipids and proteins they contain. Although insect larvae contains several macronutrients, it is essential that processing methods maintain this quality. Of the processing steps that can affect quality, slaughter is particularly important. Indeed, the slaughter method must be able to maximize the quality of the larvae while reducing the microbial load. However, some slaughter methods cause a greater or lesser stress in the larvae which can lead to the mobilization of lipids and/or promote oxidation. This work aims to compare the effect of several slaughter methods such as treatments by cold (direct freezing at 20 °C, 40 °C and liquid nitrogen for 1 h), by heat (dehydration at 60 °C for 30 minutes and boiling for 40 seconds), mechanical (high pressure processing at 600 MPa for three minutes and grinding for four minutes) and by deprivation of oxygen (negative pressure and air saturation with CO<sub>2</sub> or N<sub>2</sub> during 120 to 144 h) on lipid oxidation (TBARS; xylenol orange), microbial load (total aerobic mesophilic; *Pseudomonas* spp.; *Lactobacillus; Enterobacteriacae; Listeria* spp; coliform; *Clostridium* and other sporulated; yeasts), larval colour (colorimetry), pH, and crude lipid and dry matter content of insects. Ultimately, this project will enable the development of a technical protocol for the industry while promoting the acceleration of the marketing of insect by-products for livestock feed.

# Preliminary evaluation of an electronic counting device for rapid and precise counting of black soldier fly larvae

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The mass production of insects for human food and animal feed application is emerging internationally. The production of these insects faces several challenges in the management and automation of production processes. Larval density is a fundamental parameter to control to provide consistency in final weight and production duration. Currently, counting is performed manually or by estimating the number of larvae for a defined weight, which is very labour intensive and can lack precision, as the young larvae are very small and fragile. Our lab is working to apply an existing technology, XpertCount<sup>™</sup> currently used to rapidly and efficiently count small aquatic organisms (larval shrimp/fish, zooplankton and phytoplankton) using novel technologies based on optical image capture and analysis. Egg clutches from the Université Laval black soldier fly (BSF) colony were harvested from a 24-h period and suspended over 5 plastic containers containing Gainesville reference diet containing sodium benzoate (0.15% w:w). Incubator conditions were held constant: controlled photoperiod (12L:12D) at 27 ° C and a relative humidity of 80%. Four day old larvae were separated from the growing medium and manually counted. Groups of 1000 larvae where then introduced to the counting chamber to produce images of the larvae under varying conditions. Specific algorithms were developed to analyse larval images, and was optimised over a number of weekly counting sessions. Currently, under optimal conditions, lots of 600, 4 day old BSF larvae can be counted with an accuracy of 98%, within 5 seconds. Development of this technology continues to count significantly more larvae, as well as different stages- from eggs to pupae, as well as develop algorithms to estimate size and mass. This technology offers a strategic tool to optimize production for academic and industrial BSF production.

# Total gas and methane emissions of black soldier fly *Hermetia illucens* grown on different organic wastes

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Edible insects have been suggested as an alternative source of protein for animal feeding. The production of insect meals should have a lower environmental impact as compared to other productions such as beef or dairy products. However, few information is available in literature with special regards to Green House gases emissions. An experiment was conducted to quantify emission of gas and methane by larvae of the black soldier fly (BSF) Hermetia illucens (Diptera Stratiomyidae) grown on different organic wastes. The experimental substrates were: a standard hen diet (control), okara, maize grain dry distillers and brewers spent grain mixed with trub (derived from brewing process). An in vitro method derived by evaluation of feed for ruminants using a semiautomatic pressure system was adapted for this purpose. Twenty BSF larvae were positioned in duplicate into serum bottles and added with the experimental substrates (diets) ad libitum. For each sample two blanks (i.e. substrates without larvae) were added. Headspace pressure was recorded after 24 hours of incubation using a digital manometer (model 840082, Sper Scientific, Scottsdale, AZ, USA). The gas pressure data were converted to moles of gas using the ideal gas law. A fixed-volume sample of gas was also collected for subsequent methane analysis using gas-tight syringes fitted with needles through the bottle top. The gas composition of the headspace was determined by micro GC gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). An external standard mixture of CO<sub>2</sub> and CH<sub>4</sub> was used for instrument calibration. For all substrates, no detectable traces of methane were determined in the analysed air samples. There were differences on total gas (ml of Gas/g of incubated dry matter) produced by BSF larvae incubated with the different substrates and corrected for the blank gas production. Particularly, total gas productions were higher for larvae incubated with hen diet and brewers mixed with trub, intermediate for okara and lower for larvae incubated with distiller grains. The method proposed seems promising to estimate gas and CH<sub>4</sub> productions of BSF larvae in a simply and fast way. The differences in gas production between BSF larvae fed with the different substrates could be useful to better define the optimal diets for this species.

# Dynamic succession of black soldier fly (Hermetia illucens) gut microbiota during metamorphosis

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Black soldier fly (BSF), Hermetia illucens, develops on organic wastes, reducing pollution, and converting waste into high protein feed that can replace increasingly expensive feed of poultry, aquaculture, and livestock. The gut microbiota of BSF impacts larval growth and development. Bacteria isolated from the gut of BSF are antibacterial, able to digest cellulose, as well as degrade tetracycline. This study explored changes in the gut bacteria community richness and diversity associated with different developmental stages of the BSF. Results showed the number of aerobic-culturable microbes in BSF gut increased gradually throughout the larval phase (except for the molting process) until the maximum of 13 days larvae  $(1.90 \times 10^7)$ . The number of aerobic-culturable microbes in the gut of the middle and late pupal stages were significantly lower than in the early pupae. Furthermore, the number of aerobic-culturable microbes in early pupae (<24 h) were significantly greater than in the middle and late pupae. In addition, it was also found that the number of aerobic-culturable microbes in the process of molting was lower than that in the larvae before and after molting. In the newly emerged adults (<3 h), no aerobic-culturable microbes were found in the body, and a large number of aerobic-culturable microbes were obtained in adults after emergence for 5 days. The number of aerobic-culturable microbes in the females' gut  $(2.05 \times 10^5)$  was significantly higher than that in the male gut  $(4.50 \times 10^4)$ . The microbiota of the developmental stages were investigated by targeted 16S rRNA gene (V3–V4 region) sequencing using the Illumina MiSeq. There were 10 bacterial phyla and unassigned bacteria, four phyla of bacteria – Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria – were present in all the developmental stages. Proteobacteria was the predominant phylum in all the developmental stages. This experiment revealed the dynamic succession rule of gut microbiota of BSF at different developmental stages, and laid a foundation for further exploring the influence of gut microbiota on the growth, development and physiological and biochemical effects of BSF.

# Antibacterial activity of *Hermetia illucens* against pathogen naturally present in the pig manure and its mechanism

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Black soldier fly (*Hermetia illucens*) larvae are often associated with kitchen waste, spoiled feed, manure, and garbage. These resources are often in close contact with pathogenic microorganisms, which could contaminate black soldier fly larvae and resulting adults. These interactions could stimulate the synthesis of the antimicrobial peptides by the larvae while feeding on these resources. To investigate the molecular scavenging capabilities of black soldier fly larvae, approximately 7,500 black soldier fly larvae (seven-days old with the same weight) were added to 5 kg fresh pig manure. Manure samples were collected every two days from day 0 to day 10. In conjunction, larvae were sampled at the same time. Bacteria populations were determined for the manure samples and the relative AMP genes expressions were evaluated. Results indicate a significant reduction in pathogenic bacterial count, and this result coincided with the qPCR experiment findings. Incubation the black soldier fly larvae in pig manure stimulates an immune response by activating DLP4 and Cg-Ubiquitin expression which resulted in remarkable fall in bacterial population. Our results suggest antimicrobial substances produced by the black soldier fly larvae play a pivotal role in blocking the bacterial community viability. This may provide the basis for further combinatorial studies with *H. illucens*-derived AMPs.

# The effect of the egg associated bacteria on the hatching of Hermetia illucens eggs

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Black soldier fly (BSF), *Hermetia illucens* (Diptera: Stratiomyidae) is a large fly of great economic importance as it can be used to recycle waste to produce protein. This study aimed to investigate the impact of BSF egg-associated bacteria on egg hatch rate in order to provide some theoretical basis for large scale breeding. Results indicate the egg hatching rate decreased significantly after surface disinfection with only about 30% hatch. When mixed bacteria strains isolation from eggs (24 h) were inoculated on fresh eggs (nature and sterile eggs), successful hatching occurred. *Bacillus subtilis* and *Staphylococcus saprophyticus* were determined to significantly impact egg hatch. To sum up, the results provide evidence that egg associated bacteria can stimulate hatching of BSF eggs. The research findings contribute new insight into an important aspect of the hatching biology of BSF and providing the basis for a new paradigm of environmental factors involved in egg hatching. It is of great significance to follow-up study the effect of specific microorganism on the hatching of the eggs and to promote the sustainable development.

# Influences of bacteria on the conversion efficiency and nutrient accumulation of *Hermetia illucens* in chicken manure

Poster 38

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Black soldier fly larvae (BSFL) conversion of chicken manure could reach around 50% by manipulating symbiotic bacteria. We isolated six bacterial strains from BSF eggs, and one strain from the BSFL gut. These bacteria were inoculated as isolates as well as a communities at different concentrations in chicken manure and then fed to BSFL. BSFL highest weight gain was 28.6% when the symbiotic bacteria communities consisted of the following ratio, FE01:FE04:FE08:BSF-CL=4:1:1:1. Greatest manure reduction reached 52.9% when the symbiotic bacteria community consisted of the following ratio, FE01:FE04:FE08:BSF-CL=4:1:1:1. Greatest manure reduction reached 52.9% when the symbiotic bacteria community consisted of the following ratio, FE01:FE04:FE08:BSF-CL=1:1:1:2. Symbiotic bacteria also could influence the nutrition construction of BSFL after conversion. The strain isolated from the larval gut could help increasing the protein content 10.5% in BSFL compared to control. Amino acids content could reach highest when the symbiotic bacteria ratio at FE01:FE04:FE08:BSF-CL=1:1:1:1. Fat content and some fatty acids such as eicosadienoic acid (C20:2) and docosadienoic acid (C22:2) could be increased significantly by symbiotic bacteria group FE01:FE04:FE08:BSF-CL=4:1:1:1. Overall, we showed how companion bacteria of BSFL improved manure conversion and contributed to nutrient accumulation in resulting larvae.

# Metamorphic remodelling of the larval midgut in Hermetia illucens

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The black soldier fly (BSF), Hermetia illucens (Diptera: Stratiomyidae), is studied for its bioconversion ability of organic waste. Moreover, the high nutritional value of prepupae and pupae makes this insect useful for the production of feedstuffs. Despite the great interest for this species, data on BSF biology remain scarce and basic biological studies on BSF are limited. In particular, no information on the morphology, physiology and development of the midgut, which is implicated in food digestion and nutrient absorption, is available in the literature. In the present study we performed a structural and functional characterization of the midgut during the larva-pupa and pupa-adult transition. We also investigated how this organ changes during metamorphosis, a critical phase that, in holometabolous insects, leads to the rearrangement of different larval organs. To this aim, we analysed the morphology of the midgut epithelium, the behaviour of intestinal stem cells and the mobilization of long-term storage molecules, i.e. glycogen and lipids. Moreover, we performed experiments to investigate the functionality of this organ during the remodelling process. Our results demonstrate that, similarly to other holometabolous insects, the larval midgut of *H. illucens* is completely removed during metamorphosis and a new pupal-adult epithelium is progressively formed by the proliferation and differentiation of stem cells. During this process, glycogen deposits and lipid droplets are depleted. Moreover, functional experiments suggest that the newly forming epithelium is endowed with digestive capabilities. This work was supported by Fondazione Cariplo (Insect bioconversion: from vegetable waste to protein production for fish feed, ID 2014-0550). D.B. is partially supported by a grant from Consorzio Interuniversitario per le Biotecnologie (CIB).

# Anti-protease activity in fish intestinal homogenates is correlated to proximal composition of black soldier fly meals

Poster 40

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High inclusion levels of chitin from insect meals in fish diets have been reported to reduce growth performance and to reduce protein digestibility. However, previous work from our lab has demonstrated that the negative effects of insect meals could be higher when produced from larvae at early-stages of development, and thus having lower chitin contents. In a first attempt to identify and characterize protease inhibitors in insect meals using fish intestinal homogenates, the aim of this study was to explore the correlation between the proximal composition of black soldier fly meals and the in vitro inhibition of digestive homogenates in rainbow trout and tilapia. Total digestive enzymes were extracted from the proximal intestine of juvenile rainbow trout and tilapia, and incubated with increasing levels of homogenized fly meal. Fly meals were obtained from black soldier fly larvae fed on a Gainesville diet for 5, 10, 15, 20, 25 and 30 days post hatch (28±2 °C; 50% RH). Larvae were analysed for dry matter, ash, protein chitin, energy, lipids and fatty acid profiles using standard methods. Anti-protease activity (expressed as the % of inhibition at 150 µg of BSF meals) was found to be inversely correlated to the contents in dry matter ( $r^2$ =-0.69), chitin ( $r^2$ =-0.59) and lipids ( $r^2$ =-0.84), and especially to the contents in lauric ( $r^2$ =-0.75) and myristic acids ( $r^2$ =-0.92 and -0.87 in trout and tilapia, respectively). Conversely, protein contents in BSF were found to be highly and positively correlated to anti-protease activity in both species (r<sup>2</sup>=0.97 and 0.89 in trout and tilapia, respectively); higher protein contents being observed in fly meals produced from larvae at early-stages. Our results suggest that the presence of a protein and/or protein complex could be one of the causal factors involved in the reduction of growth performance in fish fed with insect meals. SDS-page characterization will allow us to further identify and characterize putative anti-proteases in black soldier larvae. Our study highlights the need to formulate insect-based diets that take into account the appropriate larval stages of development of the insect to meet the digestive requirements of fish species and improve growth performance.

# Effects of exposure Tenebrio molitor larvae to zearalenone

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Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by the fungi Fusarium culmorum and Fusarium graminearum, which are commonly found in the soil in temperate and warm countries and are frequent contaminants of cereal crops worldwide. The aim of this study was to assess an impact of the ZEN exposure on oxidative stress indicators and ZEN accumulation in the *Tenebrio molitor* larvae. The lipid peroxidation levels was evaluated by determining the malondialdehyde (MDA) concentration, proline (PRO) and glucose (GLU) content as a oxidative stress marker, and catalase (CAT), peroxidase (POD) activities were used as enzymatic indicators of the oxidative stress. MDA, PRO, GLU content, and CAT and POD activities were investigated using spectrophotometrical method. ZEN level were investigated using HPLC method. Results were analysed using unpaired Student's t-test and regarded as statistically significant at P<0.05. In the ZEN-treated larvae we were found ZEN and, the MDA and GLU content and the POD activity were significantly higher compared to the control group. To better assess the oxidative stress in the ZENtreated larvae, we determined the potential antioxidant activity as the ratio of POD activity to the MDA content, and the potential oxidative stress level as the ratio of the MDA content to the POD activity. In the ZEN-treated larvae, the highest antioxidant activity correlated with the lowest oxidation level, and lower antioxidant activities corresponded with the higher values of oxidative stress and consequently reduce the quality of protein and fat. In conclusion, ZEN present in the contaminated diet may induce oxidative stress in T. molitor larvae and thus decrease its nutritional value In addition, ZEN accumulates in larvae tissues.

# Acrylamide induces oxidative stress in Tenebrio molitor larvae

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*Tenebrio molitor* larvae are the well-known source of protein and lipids. Acrylamide (AA) is a toxic compound frequently used in multiple industrial and manufacturing processes, and it has been classified as toxic to both humans and animals. Insects are protected by antioxidative mechanisms responsible for scavenging reactive oxygen species which cause oxidative stress. In the current study, we evaluated an influence of the AA exposure on the enzymatic and non-enzymatic oxidative stress indicators in the tissues of the *T. molitor* larvae. Spectrophotometrical methods were used to determine catalase (CAT) and peroxidase (POD) activities, concentration of proline (PRO) and glucose (GLU), and the extent of lipid and protein peroxidation measured as concentration of malondialdehyde (MDA) and sulfhydryl groups (-SH). Results were analysed using unpaired Student's t-test and regarded as statistically significant at P<0.05. In the AA-exposed larvae CAT and POD activities were significantly lower, what then most likely resulted in the observed significantly higher levels of MDA, -SH groups, PRO and GLU as compared to control larvae. To better assess the oxidative stress in the AA-exposed larvae, we have determined the potential antioxidant activity as the ratio of the POD activity to the MDA content, and the potential oxidative stress level as the ratio of the MDA content to the POD activity. In the AA-exposed larvae the highest antioxidant activity and stress marker correlated with the lowest oxidation level. In conclusion, AA present in the contaminated diet may induce oxidative stress in *T. molitor* larvae and therefore decrease its nutritional quality.

# Effects of diet on larval development of *Zophobas morio* (Coleoptera: Tenebrionidae) in controlled conditions

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*Zophobas morio* (Coleoptera: Tenebrionidae), also called super mealworm, is considered a very important nutritional alternative in animal feed. It is characterized by being rich in proteins, high in fat and providing sufficient amounts of essential amino acids. It also contains a variety of carbohydrates, vitamins, phosphorus and many minerals. However, the percentage content of the components of the insect body will depend on biotic factors (food). This contribution provides important information of the effect of diet on the larval development of *Z. morio* and therefore on the continuity of subsequent generations and their massive breeding. To carry out this goal, the insects were reared in laboratory conditions ( $25\pm5$  °C,  $50\pm10\%$  relative humidity). Under these controlled conditions, different proportions of proteins and carbohydrates were developed and tested: (1) 25% protein + 75% carbohydrate; (2) 20% protein + 80% carbohydrate; and (3) 17.5% protein + 82.5% carbohydrate. No significant differences were found in the growth performance of super mealworm, however, the body weight gain and feed conversion ratio were relatively better in treatment two. Assuming an optimal diet with a 1:4 protein/carbohydrate proportion, the key to future research would be to determine the type and origin of protein and carbohydrate suitable for the mass production of this insect.

# Effects of diet on larval development of *Tenebrio molitor* (Coleoptera: Tenebrionidae) in controlled conditions

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In last years, insects have received increasing interest as sources of protein for animal and human food. *Tenebrio molitor* (Coleoptera: Tenebrionidae), also called yellow mealworm, is considered one of the best options because is rich in protein, easy to breed and feed. This species of insect can feed on different types of organic waste, this plasticity can be used to determine the desired nutritional composition of the resulting raw material. For this reason, it is very important to carefully select the source for *T. molitor* development. It is known that the quality and quantity of food available can affect the development of insects, in the case of *T. molitor* the macronutrient balance influences the conversion efficiency of the larvae, a critical parameter that affect the performance of the environment system. In the present work, a diet with different proportions of proteins and carbohydrates was developed and tested, the objective was to determine the optimum nutrient mixture for the massive breeding of *T. molitor*. The diets tested were: (1) 25% protein + 75% carbohydrate; (2) 20% protein + 80% carbohydrate; and (3) 17.5% protein + 82.5% carbohydrate. The larvae of yellow mealworm have a preference for diets with high protein content, so their body weight gain and feed conversion ratio are clearly benefited in treatment one. Considering that the fitness of this insect is significantly affected by changes in the proportions of food, the key to the mass production of yellow mealworm is to find the balance of the different ingredients of the diet minimizing the negative impact on the environment.

# The influence of bisphenol A of the Tenebrio molitor larvae

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Bisphenol A (BPA) is widely used in industry, and is also a well-known environmental pollutant involved in production of the reactive oxygen species. Ubiquitous presence of BPA in human and animal diets has negative effect on their nutritional value. We evaluated an impact of the BPA exposure on the oxidative stress indicators in the tissues of the Tenebrio molitor larvae. The lipid and protein peroxidation levels were evaluated by determining the malondialdehyde (MDA) and sulfhydryl group (-SH) content, and catalase (CAT) and peroxidase (POD) activities were used as enzymatic indicators of the oxidative stress. Proline (PRO) and glucose (GLU) were determined as a stress marker. MDA, -SH group, PRO, GLU content, and CAT and POD activities were investigated using spectrophotometrical method. BPA level was investigated using HPLC method. Results were analysed using unpaired Student's t-test and regarded as statistically significant at P<0.05. In the BPA-treated larvae we were found BPA and, the MDA, PRO, GLU content and the POD activity were higher compared to the control group. To better assess the oxidative stress in the BPA-treated larvae, we have determined the potential antioxidant activity as the ratio of POD activity to the MDA content, and the potential oxidative stress level as the ratio of the MDA content to the POD activity. In the BPA-treated larvae the highest antioxidant activity correlated with the lowest oxidation level, and lower antioxidant activities corresponded with the higher values of oxidative stress and consequently reduce the quality of protein and fat. In conclusion, BPA accumulates in larvae and induce oxidative stress in T. molitor larvae and thus decrease its nutritional value. Supported by KNOW (Leading National Research Centre) Scientific Consortium 'Healthy Animal – Safe Food', decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.

# Cricket farming for food security in refugee settings: a pilot study in Kakuma, Kenya

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Kenya is hosting the largest refugee camps in Africa, and displaced people will remain challenged by food and nutrition insecurity. Innovation and solutions to address refugees' food security and other needs are urgently needed. Insects are characterized by high efficiency in producing high-quality animal-source food with the use of very little resources (feed, water and space).Insect farming could be a completely new opportunity for creating sustainable livelihoods and addressing malnutrition in the resource restricted and often very harsh environments of refugee settings. We conducted a survey in 2016 among ethnic groups of refugees in the Kakuma refugee camp and found ethnic groups with no history of consuming insects, while others had tradition for insect consumption. A test trial exposing refugees to biscuits containing 10% powdered cricket showed high acceptability, also among refugees with no history of insect consumption. A pilot study of implementing cricket farming in Kakuma refugee camp was initiated in 2017 by the organization DanishChurchAid (DCA) in collaboration with GREEiNSECT partners. It was the first attempt ever for insect farming among refugees. The successful scaling up of cricket farming requires research in adopting the farming management to the environmental conditions, identifying local feed sources and documenting impacts on food security and living conditions of displaced people.

# Growth performance of house cricket (*A. domesticus*) and field cricket (*G. bimaculatus*) fed on agro-by-products

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This study investigated the growth performance of *Acheta domesticus* and *Gryllus bimaculatus* fed on different agroby-products: rice bran+ blood meal (RBBM), rice bran + spent grain (RBSG), & + rice bran + spent yeast (RBSY) and Grower's mash (GM) as the control. Mean adult weight of *G. bimaculatus* were  $0.746\pm0.05$  g,  $0.404\pm0.03$  g,  $0.0425\pm0.04$  g and  $0.412\pm0.04$  g for cohorts fed on GM, RBSY, RBBM and RBSG respectively while for mean adult weights of *A. domesticus* were  $0.271\pm0.03$  g,  $0.155\pm0.02$  g,  $0.135\pm0.02$  g and  $0.181\pm0.02$  g for cohorts fed on GM, RBSY, RBBM and RBSG respectively while for mean adult weights of *A. domesticus* were  $0.271\pm0.03$  g,  $0.155\pm0.02$  g,  $0.135\pm0.02$  g and  $0.181\pm0.02$  g for cohorts fed on GM, RBSY, RBBM and RBSG respectively. ANOVA results revealed that both species and treatment affected growth rate of the crickets significantly (*P*<0.001). The results of the study indicated potentiality of agro-by-products as a cheap source of cricket feed. By implication, the results call for evaluation of bioavailability of the ingested feed and calculation of feed conversion ratios to establish efficiency of the feeds.

# Prebiotics potential of chitin derived from farmed crickets: a gateway to improved gut health?

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To assess the prebiotic potential of chitin, ability of beneficial bacteria to metabolize chitin in growth culture was determined using different concentration of chitin. Samples were drawn from the batch culture, serially diluted and incubated to access microbial concentration changes over time. Acid production by the beneficial bacteria monitored through change of pH and end products of fermentation tested for ability to inhibit growth of pathogenic bacteria. Chitin increased the population of bacteria with potential beneficial probiotic effect. Inhibition zones from probiotic bacteria against Salmonella typhi was significant at P<0.05. Cricket chitin is a good prebiotic candidate which needs further research.

# Exploring the host plants of the edible grasshopper, Ruspolia differens, in Uganda

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The edible grasshopper, *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae), is an important source of food for humans in Uganda where they are harvested with powerful lights from the natural populations during the two annual swarming seasons. Despite its high economic and nutritional value for humans, the biology and ecology of this species is poorly understood. We explored the host plants of *R. differens* with laboratory experiments (no-choice and multiple-choice experiments) and a 15-month field monitoring study of a non-swarming population in Uganda. Based on the laboratory experiments, *R. differens* accepts a wide range of grass and sedge species but has a clear preference for certain host plants, and inflorescences, in particular. Based on the field monitoring study, the most important host plants at our Ugandan study location were grasses *Brachiaria ruziziensis* R. Germ. & C.M. Evrard, *Panicum maximum* Jacq., and *Chloris gayana* Kunth. Understanding of the host plants, and their preferred parts, is essential when selecting the appropriate foods for the small-scale mass-rearing of *R. differens* in the future. Such rearing methods could help prevent overexploitation of natural populations of *R. differens* in the long-term and to enhance food security in East Africa. Furthermore, the conservation of viable natural populations of *R. differens* in the long-term is only possible if grasslands with the acceptable hosts are available in East Africa.

# The present conditions of edible insects in Japan and future issues

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Even those who feel resistance to eating insects know there are countries that have such cultural practices. About 100 years ago the insect food culture was broadly rooted in Japan. However, the number of people who recognize insects as food has decreased primarily to individuals in Nagano and the inland district. The Food and Agriculture Organization of the United Nations (FAO) report published in 2013 has been reviewed by various media outlets in Japan including TV and newspapers. It is expected that insects will become an accepted food in the near future as such food streams could prevent starvation while also reducing obesity problems. However, the basic appearance of insects is one of the factors to reduce an appetite from loathsomeness with grotesquerie. We are trying to change this impression of insects as food. In order to do so, we hold an edible insect event every month. We prepare dishes using insect powder (cricket and meal worm) that are sold on the market. We would like to reduce the negative image of insects as food and make edible insects more the sustainable society edible insect was prolonged to support nutritional is, we believe that it is important to be actively used in everyday menus.

# Factors influencing willingness to pay for insect-based feeds among smallholder fish, pig and poultry farmers in Kenya

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The urgency to find an alternative livestock feed ingredient for fishmeal and soybean meal, which represent 60-70% of production costs has led to market recognition of insect-based proteins as a viable option worldwide. Being a novel source of protein, there is lack of information on the key drivers of adoption, which include the farmers' knowledge, perception, practices and willingness to pay (WTP) to facilitate commercialization. The present study assessed farmers' perceptions and WTP for insect-based feeds, and further evaluated the potential influence of different factors on the WTP estimates. A household survey was conducted among 957 randomly selected poultry, fish and pig farmers in four Counties: Nyeri, Kiambu, Kakamega and Uasin Gishu. The results revealed that 80, 72 and 68% of poultry, fish and pig farmers, respectively, used commercial feeds. In addition, over 70, 80 and 65% of poultry, fish and pig farmers, respectively, were aware that insect-based meal could form an integral part of livestock feed. Results of the regression analysis showed that age, availability of treatment, agricultural inputs, educational level, household size, positive attitude of insect as a good source of feed ingredient, male respondent, use of feeds, availability of training and availability of market information had a positive influence on WTP for insect-based feeds. Based on the findings, there is need for increased extension services to educate and promote the use of insects among communities, access to micro-credit and availability of market information to farmers to help increase adoption rate of the insect-based technologies and promote commercial feeds manufacture. As distance to feed market was found to negatively influence the WTP, stakeholders should thus target developing distribution networks that favour accessibility by end users situated away from the urban centres.

# Entomophagy, urbanization, and livelihood commodification: a case study from Naivasha, Kenya

Poster 52

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Urbanization and livelihood commodification are leading causes of socio-cultural and environmental change across Africa. This change involves also traditional practices of insect consumption in nuanced and contextualized ways. What happens to these practices when people, formerly gathering and consuming insects in a prevalently subsistence-oriented economy, migrate to distant towns and engage in a salary-based livelihood? We address this question using the Lake Naivasha basin (Kenya) as a case study. In the last twenty years, towns in the area have swollen following waves of internal migration that brought together tens of thousands of people from diverse ethnic backgrounds, attracted by the possibility of employment in the flower and vegetable farms surrounding the lake. Through semi-structured interviews with these migrants, we explored the dynamics of insect consumption following urbanization and livelihood commodification. Foremost, moving from wet tropical areas to semi-arid urbanized highlands (Naivasha is a volcanic lake with a surface elevation of 1,900 metres), migrants hardly continued to harvest traditional edible insects due to the very limited presence in the new environment. As a consequence, some migrants discontinued their use of specific insects: migrants from Western Kenya abandoned the consumption of the Gonimbrasia caterpillar, also due to cultural reasons such as the association of caterpillars' consumption with 'backward' practices in the new cultural environment. Others maintained traditional insect consumption only when travelling back to the place of origin, associating to these foods elements of cultural identity and sense of belonging (e.g. the consumption of the long-horned grasshopper *Ruspolia differens* Serville, considerd a delicacy by ethnic groups of the Lake Victoria region). Still for other insects, migrants established informal trade and gift chains from their place of origin (e.g. Macrotermes species are often brought to Naivasha following workers' trip back). Naivasha is a microcosm of the dynamics involving insects consumption following consumers' migration, urbanization and livelihood commodification. These dynamics vary in accordance to insect species, cultural relevance, and the place and context of migration, and range from the discontinuation of insect consumption to the establishment of commercial and gift networks that provide migrants with insects. Studying these dynamics can provide insights into the cultural and social aspects of entomophagy, as well as background information for the establishment of short and sustainable market chains that can provide workers in towns with nutritious and culturally-relevant food.

# Exploring the ethno-entomology of the Babukusu community in western Kenya

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Edible insects provide a good source of protein and micronutrients. At least 113 countries are documented to routinely practice entomophagy. We discuss how the traditional food base of insects for the ethnic group Babukusu in Kenya is being eroded. The Babukusu are one of the seventeen Kenyan tribes of the Luhya Bantu who live in Bungoma County, western Kenya. Entomophagy was common among the Babukusus and played a significant role in the diversity of the food system and diets. However, Babukusus eat ugali (maize is a staple food) and consider meat and chicken as a superior accompaniment. Purposive sampling of 13 respondents aged over 80 years took place in the Sikhendu village of Kimilili. Through in-depth interviews six women and seven men responded to a checklist and a semi structured questionnaire. They described the insects' seasonality and methods of preparation. Associated folklore stories were also told. The five common and most commonly consumed insects were the dung beetle, army worms, grasshoppers, termites and ants. Traditionally these insects were considered a delicacy and a preferred meal accompaniment when in season. During the rains there would be the flying termites (reproductives) known locally as 'chiswa' and also the 'door head' ants (Carebara spp) of which the vernacular name is 'kamachichi'. After the rains grasshoppers known as 'chisike' or 'kamatete' are celebrated for enhancing fertility. Army worms 'chikhungu' are blanched then stewed with soda ash to tenderize it; in this way it accompanied the ugali. Dung beetle larvae, named 'kamasibili' are available in manure throughout the year. Traditionally they were a routine part of the diet. The Babukusu have a profound knowledge of a variety of edible insect species and their contribution to health in order for the communities to better develop their resources. Insects should now be reared to assure a constant supply.

# Multiple and sustainable uses of termites and termite mounds in southern Africa and South-East Asia

Poster 54

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The termite is one of the major food insects in the world. The collecting techniques and cooking is varied. The soil of the termite mound is used in various ways. This study not only emphasizes the importance of termites as food. It also indicates the multiple uses of termite mounds for sustainable subsistence and farming. The data were collected by field work and surveys were conducted in southern Africa (Botswana, South Africa and Zimbabwe) and South-east Asia (Cambodia, Lao PDR, Thailand, and Vietnam). The methods of collecting and cooking depends on life stages. Since 1993, we investigated the use of termite mounds including soils and the products from specific plants on termite mounds. In southern Africa, the workers, solders and reproductive are used for food. They are boiled and dried in the sun for preservation and sold at the markets. The soil of the termite mounds is used as material for bricks and flooring of the house, and as fertilizer for maize. The soil is eaten in particular by pregnant women for micro nutrition intake. The termite mound is used as a field to grow crops. The mound is used sustainably, and it can become as large as 20 m in diameter and 7 m in height. The mound is a sacred place especially for ancestors. In South-east Asia the reproductives are used for food. The termite mounds are found in the paddy fields. The smaller mounds are sometimes used for planting vegetables. The larger mounds with tall trees are kept in the paddy field and has an effect on rice production; this is a special landscape composed of paddy fields and woodlands in a mosaic. In Thailand the soil of the termite mound is used as fertilizer for celery. However, the farmers do not keep the mounds for sustainable use and sometimes the mounds are regarded as sacred places.

# Exhibition design to reduce disgust for eating insects

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The habit of eating insects is disliked by many people who have no customs. We held feature exhibitions on eating insects in Itami city museum of insects in Japan during 2007 and 2015 with the aim of identifying frequency of disgust when approached with the topic of eating insects and to educate people about such opportunities. The residents in the area around the museum do not have the custom of eating insects, and many of them do not want to eat insects, some also do not know that insects can be eaten. These exhibitions aimed at getting people to know the culture of eating insects as a relationship between human and nature. In order to do so, we displayed various cooked insects from all over the world, tools of collection and rearing edible insect, and some topics on eating insects. In order for visitors to have familiarity with eating insects, we implemented several approaches. For example, in exhibits of cooked insects we displayed not only insects, but also photos of eating people, and some captions showing impressions of eating each insect by the exhibition curator who was from the same area with the museum's visitors. Some of the visitors were surprised, but many visitors watched with interest.

# Application of emerging technologies to transform and valorise insects according to the biorefinery concept

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Satisfying the growing demand for proteins, notably for the purpose of feeding animals, and toning down the impact of animal husbandry on the environment, while at the same responding to public demand for product quality, are major challenges for international organisations, private operators and agri-food researchers. Among several possible additional sources of proteins, the insect solution seems to be relevant and credible as a complementary option alongside other conventional ones (fish and soya). The development of an insect industry requires the development of technically reliable and economically viable transformation processes. In this work larvae of *Tenebrio molitor* were used for investigation. These larvae are rich in water, proteins and lipids. The objective of the study was to fractionate the raw material in order to obtain a liquid fraction rich in oil and a solid fraction rich in proteins according to the biorefinery concept. For this purpose, mechanical expression was used for insect dewatering and defatting. The experiments were conducted using a hydraulic (discontinuous) and a screw (continuous) press. In order to enhance the pressing kinetics and to increase the extraction yield, different pre-treatments were used. These treatments include conventional (bleaching in hot water) and novel technologies (pulsed electric fields, ultrasounds and microwaves). The obtained products were analysed. Results showed that the application of a pre-treatment before pressing improves the extraction yield and reduces the extraction time. Pulsed electric fields seems to be very promising technology. In fact the application of the electrical treatment induces cells membranes permeabilization facilitating thus the extraction step at cold temperature and preserving the product quality. The results obtained in this work will be very useful to pave the way for insect's valorisation in the biorefinery concept.

# Optimization of freeze substitution and inclusion with glycol methacrylate for histological studies of black soldier fly

# \${protocol}://www.wageningenacademic.com/doi/pdf/10.3920/JIFF2018.S1 - Wednesday, May 30, 2018 1:34:27 AM - Universita' Statale di Milano IP Address:159.149.162.67

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The industrial application of black soldier fly (BSF) larvae (*Hermetia illucens*) has received significant research attention recently due to its potential for mass rearing on waste organic residues and applications ranging alternative animal feed ingredient to antimicrobial peptides. However, many morphological and physiological features of the black soldier fly larvae have been mostly absent from the literature. One such feature is the digestive tract and associated organs; it has not been documented how the digestive tract anatomy develops or how this adapts to various feed sources. We present herein a protocol describing the fixation, dehydration infiltration and polymerization of whole black soldier fly larvae using a freeze-substitution technique and processed in glycol methacrylate (GMA). Larval BSF were grown on Gainesville reference diet (30% dry matter, 28 °C; 60% relative humidity), and collected at 4, 6, 8, 10 days post hatching. Larvae were flash frozen in melting isopentane (-160 °C) for 2 minutes. Larvae were then processed at -30 °C in graded levels of precooled acetone and GMA using single or multiple solvent/GMA changes with 2 final changes of 100% GMA. Processed larvae were polymerized in 12×8 mm blocks overnight under vacuum in the absence of oxygen. Unmoulded blocks were sectioned (3 µm thick) using a glass knife-equipped microtome. Sections were floated on water, dried, stained via Meyer's hematoxylin and eosin, and subsequently mounted in Permount. Tissue conservation and integrity were assessed and compared using formalin/wax embedded control sections. Flash freezing and freeze substitution offers a flexible approach to ensure conservation of formalin and heat-labile targets. The optimized processing parameters will permit application of a range of histological approaches, including enzyme histochemistry and immunochemistry.

### Crop straw utilization by the blow fly Chrysomya megacephala

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Crop straw is an abundant renewable resource; however, there is limited usage for crop straw due to the high content of cellulose, hemicellulose, and lignin involved. Thus in the present study, we crushed and fermented corn straw to formulate artificial diets for rearing the blow fly *Chrysomya megacephala*. The suitable formula for mass rearing of the blow fly was as follows. Corn straw was first fermented for 3 days and then mixed with wheat bran at 1:1 ratio by weight, and 45 mg eggs were inoculated in 200 g larval diet (water content: 75%). Blow fly larvae developed well over the diet with the lowest feeding cost. We harvested 13.4 g mature larvae and 40.4 g residue. Protein and crude fat content of resulting blow fly larvae were  $64.74\pm0.11$  and  $17.28\pm3.88\%$ , respectively. Meantime, the content of soluble sugar, cellulose, hemicellulose, and lignin in straw diets significantly decreased after larval feeding which ended up with 9.0, 11.0, 21.4 and 19.8% respectively. We also evaluated the index of corn straw diet residue for developing organic fertilizer. It showed that the pH of residue was 7.1 and the total nutrient content (N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O) and the organic matter content of the residue were 6.4 and 91.3% respectively, which correspond to the China organic fertilizer standard (NY525-2012). The content of heavy metals including Cd, Cr, Pb, As, Hg, were far below the national standard. Our results provide the promising crop straw utilization model transformed by insects. This study was funded by Agricultural public welfare industry research from Ministry of Agriculture of People's Republic of China (201503137).

# Effects of corn straw fermentation by *Trichoderma viride* and yeast on rearing *Musca domestica* (Diptera: Muscidae)

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China is one of the largest crop straw producing countries in the world. As a by-product of agricultural, crop straw is also an important biological resource. It is urgent to develop new methods to utilize crop straw efficiently in China. Here we report that crop straw was fermented with the aid of *Trichoderma viride* and yeast as an artificial diet for rearing of the house fly, *Musca domestica*. Results suggest the absolute content of cellulose, hemicellulose, and lignin in corn and wheat straws decreased significantly after the fermentation and larval feeding. The optimum diet for mass rearing of *M. domestica* was corn straw mixed with wheat bran at the proportion 1:1. Then we added 7% T. viride and 1% yeast by weight to ferment the mixed straw for 5 days, and supplied 200 mg of newly-hatched M. domestica larvae per 250 g of diet. By using different diets to raise *M. domestica*, the conversion rate were as follows: corn straw > wheat straw > wheat bran. The highest conversion rate was 16.19% raised by corn straw, whereas the wheat bran was the lowest (8.89%). In conclusion, we provided a novel strategy for the sustainable utilization of crop straw in China, that is, fermented crop straw with *T. viride* and yeast firstly and then feed to the housefly larvae. This study was funded by Agricultural public welfare industry research from Ministry of Agriculture of People's Republic of China (201503137).

### Protein molecular spectral features in the heterogeneous feed matrix/issue with advanced vibrational molecular apectros

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Feed tissue structures are not homogeneous. Protein bodies are located in this heterogeneous tissue. The strategy to reveal the molecular spectrum of protein located inside these heterogeneous tissues is to use molecular technology with ultra-spatial resolution. Synchrotron-based microspectroscopy can identify and select the relatively 'pure' protein dominated spectrum among mapping pixels and minimize other biological components absorption and scattering contributions in feed tissue. The objective was to use the synchrotron-based microspectroscopy to determine feed protein infrared spectral features in the heterogeneous feed tissues. Protein structures can be quantified using multi-component peak modelling based on the feed amide I component band infrared absorption intensity. The results demonstrate the potential of highly spatially resolved synchrotron microspectroscopy to reveal the molecular structural-chemical makeup in heterogeneous feed tissue. Further studies are needed to quantify the relationship between the feed molecular structure features in the tissue and nutrient availability in animal models.

\${protocol}://www.wageningenacademic.com/doi/pdf/10.3920/JIFF2018.S1 - Wednesday, May 30, 2018 1:34:27 AM - Universita' Statale di Milano IP Address:159.149.162.67

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