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Preface

The INSECTA conference 2017 aims to give an overview of the state-of-the-art of insect technology and the prospects and constraints of the use of insects as food, feed and non-food in Europe and worldwide. This publication contains abstracts of all talks and posters presented at this conference.

In 2015, PPM (Pilot Pflanzenöltechnologie Magdeburg e.V.) initiated the first national INSECTA in Magdeburg held in German language. The 2nd INSECTA was jointly organised by PPM and ATB (Leibniz-Institut für Agrartechnik und Bioökonomie e.V.). To address international experts from academia and industry, English was chosen as conference language. Our aim in 2017 was to continue the success of recent congresses, 1st and 2nd INSECTA held in Magdeburg in September 2015 and 2016, and ensure that INSECTA will be the insect science and technology conference to attend in 2017 for industry and academia alike.

ATB and PPM organised the 3rd INSECTA in Berlin, Germany in September 7 and 8, 2017. The theme of the conference is “Insects as Feed, Food and Non-food – A new Bioresource”.

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

Within a broad scientific program with a number of oral sessions over the two days of the conference five central conference themes have been identified:

- ✓ Insects Production Systems
- ✓ Application of Insects as Feed
- ✓ Utilization of Insects as Food
- ✓ Evaluation of the use of insects
- ✓ Non-Food Applications of Insects

In total, the conference has attracted 84 presentations from 28 countries. In the conference period of two days, 3 keynote lectures and 50 oral presentations have been held in 3 plenary and 9 parallel sessions, with 34 posters presented in the poster session. We hope that the conference and this publication will provide the platform for bringing together those involved in insect production, processing and consumption worldwide to explore the frontiers of knowledge in the art, science and engineering of all types of processing methods, monitoring technologies, and quality management systems; and the opportunity for participants and the public to learn about the latest developments in the respective fields, to improve communication and to increase cooperation and coordination of research efforts among researchers.

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

Oliver Schlüter, Birgit Rumpold, Frank Pudel Potsdam, den 30.08.2017

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2015

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525 mio tonnes protein

35 % food

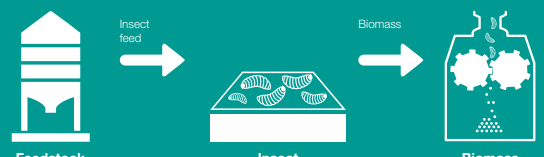
65 % feed

food

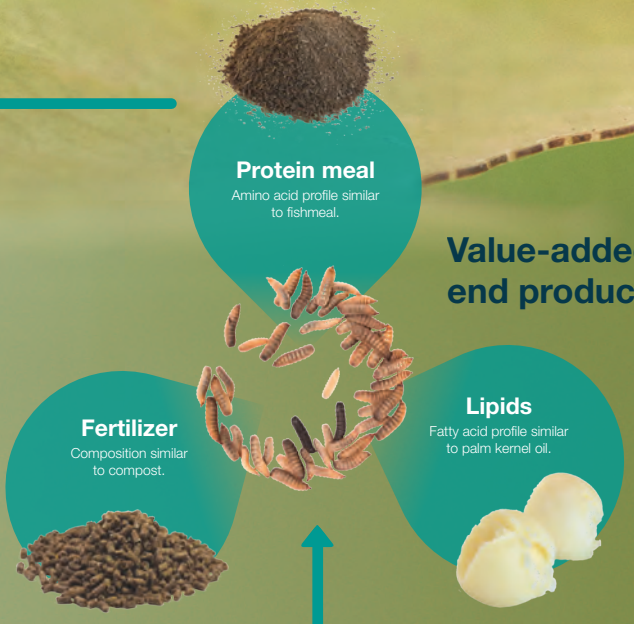
Losses & Waste

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

Insect Technology



Value-added end products



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Oral

Session 1 – 9

Food sustainability and the protein puzzle: different resources and disciplines

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In order to fully appreciate the intricate relationships between sustainability and food, the input of at least a dozen different scientific disciplines is required, including ecology, chemistry, medicine and nutrition science, as well as economics, psychology and political science. This keynote starts by identifying ecological, economic and social aspects of food sustainability and food security, and by prioritizing the environmental impacts associated with food production and consumption. Subsequently, it is argued how nitrogen and protein are underlying and linking the top-3 of anthropogenic impacts, i.e. 1) biodiversity loss, 2) nitrogen cycle acceleration, and 3) carbon cycle acceleration (a.k.a. climate change). Addressing agriculture, food industry, consumers, and governmental stakeholders, as well as cultural aspects, challenges and options are sketched, with 2030 and 2050 as important waypoints. Meeting the UN Sustainable Development Goals requires a relatively rapid transition towards a circular economy. In this light, insects are undeniably useful for food, feed, and other purposes. In the Western world, however, entomophagy has some hurdles to negotiate still. Health may be key to entice consumers to drop their conservative attitudes and progress towards a diet transition. In Asia and Africa, the situation is entirely different from EU and US. In summary, global and local trends in ecology as well as society are highlighted from the perspectives of different resources and disciplines.

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Ethical perspectives on using insects for industrial food and non-food applications

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Currently, insects are announced as solutions in food, feed and raw materials for a more sustainable production. But what are the criteria for more sustainability? It is here where ethics comes in, asking for the morally justified goals and means of human action. Usually, people do not care morally for the well-being of insects, often to the contrary. If insects are to be kept and raised for mass production, it is at least instrumentally prudent to hold them in good conditions. And beyond that, do humans have also direct moral obligations since insects are living beings and may be treated adequately? What about 'humane' forms of killing? Are there species which may not be utilised due to specific characteristics? Moral and interdisciplinary questions on the moral status of insects, their sensory and 'cognitive' abilities and ensuing rules for treatment depart, which will be discussed in the talk. Does it make sense to take the imitation of 'naturalness' as a criterion for adequate conditions for each species?

The second group of questions is to which sustainability goals insect production should serve. Is it sustainable to replace material like soy as a protein source in meat and fish production without asking the question of meat consumption patterns and issues of global food justice and food sovereignty? What about biosafety and biosecurity issues? And, although seemingly less important, what about the moral dimensions of the aesthetic 'yuck-factor', or cultural taboos? In all these respects, it makes sense to think about the moral relations between humans and insects in some more detail.

Implementation of circular economy through a holistic application of black soldier flies in waste management

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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 (for more info see http://icrofs.dk/en/research/danish-research/organic-rdd-1/bioconval/) - but during the last couple of years DTI has focused their efforts on black soldier fly (BSF) larvae 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 BSF larvae reared on organic household waste (e.g. https://www.dti.dk/projects/project-can-insects-convert-organic-household-waste-into-valuable-mink-feed/38308) as well as assessment of the potential of other high volume residuals as feed. Overall, our work with insects, and BSF in particular, 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 BSF conversion of a variety of different substrates – this will include: i) 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. ii) 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 ton of waste has been converted and the influence of seasonality in waste composition and larvae density has been investigated. iii) Assessment of BSF 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 coming out of the production. iv) Digestibility and palatability feeding trials with BSF larvae (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. v) Assessment of palatability of BSF in mink was also very promising ranging from 96-99% of all the feed being consumed. In the presentation preliminary data from a full-scale mink feeding trial will also be included.

Direct and indirect biorefinery technologies for conversion of organic side-streams into multiple marketable products – BBI-InDIRECT project

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'Management of waste as a resource' was described in a European roadmap (COM (2011/571) as a milestone to be reached by 2020. On the one hand, there are diverse under-spent side-streams situated at the farmers and post-harvesting level as well as in the associated retail sector. On the other hand, there is a need for new resources, for instance proteins as alternative for soy proteins.

InDIRECT, a three-year EU research project started November 2016, aims to develop biorefinery processes as part of new value chains to convert under-spent side-streams from the agro-sector and processing sector into useful marketable products. Cascading processes (recovery of multiple compounds from the same feedstock) are envisioned to increase the conversion efficiency and maximise the values of the feedstock. Direct biorefiney of the biomass will be compared with indirect biorefinery. The latter refers to the two-step process that can cope with the heterogeneity of side-streams. In a first step the heterogenic feedstock is converted to homogenous biomass using insects. Insects are able to convert a variety of feedstocks into a more homogenous biomass, being their own biomass. In a second step, the insect biomass is further fractionated into a lipid, protein and chitin fraction, that all three have potential towards marketable end products. The direct approach (without the insect conversion step) which is being explored for green leaves, is expected to be more seasonally variable compared to the indirect biorefinery appraoch, but generates a larger variety of compounds. The project foresees to process the derived fractions further into multiple marketable products for the feed, food and chemical sector.

The multidisciplinary InDIRECT consortium consists of 9 partners from four countries (Belgium, The Netherlands, France & Italy), comprising 2 research organisations (VITO & University of Parma), 5 industrial partners (Nutrition sciences NV specialized in feed additives, two insect farms Millibeter & Proti-Farm R&D, IMPROVE specialized in plant proteins, and CHEMSTREAM focusing on chemical application), a non-profit organisation that works in close contact with farmers and processing industry (Innovatiesteunpunt) and a project supporting company (Temperio). The project (www.bbi-indirect.eu) is coordinated by VITO.

This project has received funding from the Bio Based Industries Joint Undertaking under the European Union’s Horizon 2020 research and innovation programme under grant agreement No 720715.

Authorising edible insects under the new novel food regulation: Problematic aspects concerning the dossier submission

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Problem Statement: Before the adoption of the new novel food regulation, there was legal uncertainty on the regulatory classification of edible insects. Except for Regulation 834/2007/EC on organic production, in the European legislation no mention was expressly made to insects as food. Considering this legal gap, insects and insect-based food were considered by most to fall within the scope of the novel food Regulation (EC No 258/97), although this qualification was far to be undisputed and not consistently implemented in EU member states (PAGANIZZA 2016).

The classification of insects as novel food has been clarified through the adoption of Regulation (EU) No 2015/2283, which replaces Regulation 258/97. Recital (8) of the new regulation expressly states that "it is appropriate to review, clarify and update the categories of food which constitute novel foods. Those categories should cover whole insects and their parts". Following this review, all edible insects can be deemed to fall within the novel food category under article 3, 2 (a), (v) that encompasses "food consisting of, isolated from or produced from animals or their parts".

The novel food status of edible insects implies that they are subject to safety assessment and pre-market approval before being placed on the market. The new Regulation provides two different procedures that can be used to place edible insects in the European market. In both cases, the applicant is required to submit a set of information to the Commission that may involve the European Food Safety Authority (EFSA) in the food safety assessment.

The faster way to place edible insects on the European market is the notification procedure set forth in article 14 for traditional food from a third country. Although insects are deemed to be food without a long history of consumption in Europe, it is a matter of fact that they have been widely consumed in Asia, Africa and South America.

Whilst the notification procedure presents the advantage of requiring a lower amount of information than those required under general rules (e.g. it is not required scientific evidence demonstrating that the novel food does not pose a safety risk to human health) and a faster timing, applicants cannot require the protection of data submitted through the dossier.

Objectives: The primary goal of the paper is to analyze the authorization procedures provided by the new novel food regulation, comparing the set of information and the

level of safety evidence required under the old novel food regulation and these required by the new one. In that perspective, the guidelines issued by EFSA in September 2016 (EFSA, 2016) will be critically analysed, highlighting the aspects that appear to be more relevant when preparing an application for authorising an insect species.

In carrying out this analysis, the scientific opinion on a risk profile related to production and consumption of insects as food and feed, issued by EFSA in 2015 (EFSA, 2015) will be taken in consideration.

The paper will also explore the conditions under which the applicant can protect the information submitted in the dossier, applying for data protection under Article 26 of Novel Food Regulation. In particular the data protection provided by the Novel Food Regulation will be critically analysed through a comparison with the data protection regimes put in place under other regulatory frameworks (Health Claims Regulation, Plant Protection and Reach Regulation).

Procedures/methodologies/approaches: The methodology chosen consists of a comprehensive literature review of scientific articles published in the last decade on novel food application as well as on the legislation concerning insects as food. Regulation 2015/2283/EC and the guidelines on application issued by EFSA in 2016 will be critically analysed and commented.

Results: Preliminary results show that although the notification procedure, provided for food having a history of consumption in a third country appears to be the most viable solution for approving the consumption of edible insects, this route appears to have some limits. First of all, applicants cannot obtain the data protection for the information submitted in the dossier, meaning that – the authorisation being generic – the first applicant can not secure any competitive advantage towards the other insects producers. Notwithstanding this, the requirements under the notification procedure make it mandatory for the the applicant to specify the intended use and maximum use level(s) and concentration(s) in the final product. As a consequence, only products meeting these criteria could be placed on the European market.

As concerns the data protection, some concerns rose by the inclusion of the "exclusive right of reference to the proprietary scientific evidence or scientific data" among the conditions that need to be fulfilled.

Even though the provision on data protection does expressly preclude protection to scientific data already published, in the past the European Commission has interpreted the wording "exclusive right to reference" as precluding protection to studies which have been already published and made available to the public domain. This interpretation implies that peer- reviewed publications – which are generally considered by the scientific community as being the best scientific - cannot benefit from protection under Article 26 of the Novel Food Regulation (HOLLE 2014).

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Keywords: novel food regulation, authorisation, edible insects, dossier, food safety, European Food Safety Authority

Healthy snacks: An In-depth analysis of the dietary and therapeutic benefits of Mopane worms (Lepidoptera: Saturniidae)

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Edible insects are increasingly being recognized as food source for nutrition deficient population across the world, especially in developing countries. Among the edible insects, saturniid caterpillars: *Gonimbrasia belina* L. and *Gynanisa maja* L., which feed almost exclusively on the mopane trees, *Colophospermum mopane* (Kirk ex Benth.) are highly preferred and consumed in Southern and Central Africa. However, detailed information on the dietary and therapeutic benefits of these insects are scant. In this regard, we analyzed traditionally prepared and dried form of the caterpillars for nutritional components; therapeutics including antioxidants, amino acids, essential fatty acids and flavonoids and mycotoxins using liquid chromatography coupled to quadrupole time of flight mass spectrometry (LC-Qtof-MS) and coupled gas chromatography (GC-MS). We also performed inductively coupled plasma - atomic emission spectroscopy (ICP-AES) analysis to determine micro- and macro-nutrient concentrations in the caterpillars. The results showed that the two caterpillars have high nutritional value with crude protein content of *G. belina* (72.8% DM) being significantly higher compared to that of *G. maja* (61.3% DM). The fat levels were comparable for both *G. belina* (17.5% DM) and *G. maja* (16.4% DM). Twelve amino acids were identified in both caterpillars including Lysine and Methionine, which are the most limiting in cereal based diets. On the other hand, we identified 10 fatty acids, which included saturated, monounsaturated and polyunsaturated fatty acids, which are considered essential for human nutrition and health. Analysis for health beneficial like anti-oxidants revealed a total of 4 major flavonoids. High levels of macro-minerals (phosphorus, sodium, potassium and manganese) and micro-minerals (iron, manganese, cobalt and zinc) were also recorded in both caterpillars. Overall, our results showed that both caterpillars are valuable nutrient – and – antioxidant-rich source of food and, provides important information to allow for the inclusion of insect-rich source of Fe and Zn in foods as a sustainable and effective way to improve human nutrition and health.

Adoption of insects as a source for food and feed production: determinants of acceptance between urban consumers from Maputo/Mozambique

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Many traditional societies have used or still use insects as a protein source, while westernized societies are reluctant to use insects, despite being the major consumers of animal proteins. Edible insects are highly nutritious with high fat, protein and mineral contents depending on the species and thus represent a noteworthy alternative food and feed source. Nevertheless, consumer acceptance needs to be established. The aim of this work was to evaluate consumer acceptance of insects as food or feed, considering consumers from urban Maputo in Mozambique. 216 consumers were interviewed, divided by gender and age group, and were asked to complete a questionnaire comprising the following constructs: i) Health, Convenience and Ecological Welfare; ii) Food Neophobia Scale; iii) Awareness towards the use of insects as food; iv) perceived acceptance of insects as food (consumption of edible insects and of proteins bars with cricket flour) and as feed (consumption of fish, poultry, pork and beef from animals fed with insects); v) Perceived acceptance of sushi; vi) Disgust towards insects as food; and vii) Knowledge regarding edible insects; complemented with socio-demographic data. Acceptance of insects as food was transformed into binary choice (0/1). Dichotomous decision was fitted using a binary logistic regression model (Health, Convenience, Ecological welfare, FNS, Disgust, Sushi acceptance, age, sex, higher education, Familiarity, Previous experience). Consumers were segmented, following Clustering Analysis, based on the perceived acceptance of the consumption of insects, protein bar with cricket flour, fish, poultry, pork and beef from animals fed with feed formulae incorporating insects. Acceptance of edible insects is strongly modulated by disgust, showing that over 60% of the participants rejected any form of entomophagy. This being lower than the one previously found in Western countries, such as Portugal and Norway. Respondents from urban Maputo also present lower acceptance of fish, poultry, pork and beef from animals fed with formulae incorporating insects or insect proteins.

These results provide insight on how consumers from urban Maputo perceive the acceptance of insects as food and feed, and stress the influence of cultural differences within a country with traditional practices of entomophagy. Possible causes for such strong rejection of insects as food and feed is the pursuit of an ideal of Western food eating patterns.

Industrialization of Insect Farming: New challenges to prevent pathogenic hazards

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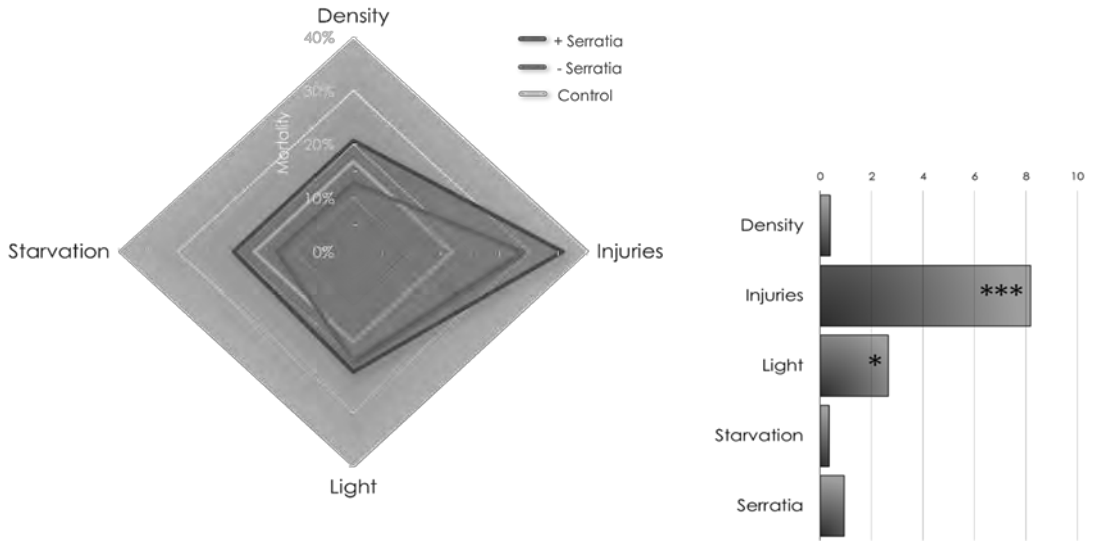
In recent years, insect farming has experienced a genuine worldwide expansion through diverse applications in biological control, chemical industries, animal feed or human food. Nowadays, the activity is developing at industrial scale projecting to produce more than dozens kilotons of insects per year. This important scale-up raises several major challenges for technological innovations but also for health and safety issues. Actually, the most damageable risk to be considered for an insect farm should be the disease outbreak. However, the knowledge of pathogens and parasites of insects is still very limited, and new pathologies emergence could also appear with the rise of insect mass production. In this domain, only millenary practices of beekeeping or sericulture benefit from extensive knowledge on prophylaxis, pathogens detection and therapeutic methods. Finally, the success of this emergent agroindustrial sector based on insect farming depends a lot on effective infectious risk management. Ynsect is especially concerned about this issue and are developing research programs, rearing units monitoring and quality procedures to prevent health hazards.

The purpose of the speech is, in a first part, to do an overview of insect farming sector through its industrialization and to discuss about the measures taken to manage infectious risks. Then, in a second part, some results are presented from a collaborative project with INRA Micalis on pathogen infectious signs detection in a *Tenebrio molitor* rearing. The study aims to identify biotic and abiotic factors that can facilitate *Serratia marcescens* infection and then to develop methods for an early detection of contaminated insects in mass production. *S. marcescens* is a well-known entomopathogen which favorably developed in insect farming (BUCHER 1963) when rearing conditions are degraded. It is also a potential pathogen for humans and animals, and so should be carefully considered in production of insects for feed and food. Several series of challenging tests were set up on yellow mealworms: (1) by direct subcuticular injection, (2) by *S. marcescens* contaminated feed ingestion (3) by co-infection with *S. marcescens* and virulent strains of Bacillus thuringiensis and *Cordyceps bassiana*, and finally (4) by abiotic factors (light exposure, starvation, density, injuries, temperature, humidity, agitation) combined with *S. marcescens* contaminated feed ingestion. As shown in the figures below, *Tenebrio molitor* larvae are resistant to most of tested biotic and abiotic stresses. From a selection of stressors, only injuries (and to a lesser extent light) lead to a significant increase in mortality from 16% to 30%, aggravated by the presence of *S. marcescens* (36% of mortality). However, the consumption of contaminated feed has few impact on the mealworms rearing. *Serratia marcescens* infection is preferentially triggered by a lesion caused by injury or attack by a second pathogen (*B. thuringiensis or*

C. bassiana). These results therefore allows to extend knowledge on pathogen infection process but also to develop molecular detection tools based on qPCR methods.



Mortality induced
from stress tests in presence or not of *S.marcescens*

In conclusion, the present studies of both the historical disease outbreaks in animal farming including insects, and the tests on insect resistance to pathogen demonstrate that infectious risk prevention is essential for the raise of "entomo-industry". Several axes must be taken into account, such as (1) the choice of the reared species (we show here the good resistance of *Tenebrio molitor*), (2) the establishment of a prophylactic system integrating biosecurity principles, (3) the early detection of risk factors looking at both the pathogenic infections appearance and the abiotic causes, and finally (4) the development of knowledge and technics in insect health.

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Exploiting insects as feed for sustainable salmon farming - identifying the risks of pathogen transfer within the production chain

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The global aquafeed industry traditionally relies on fishmeal for protein in the diets of farmed fish. Concerns about overfishing have seen a shift towards greater inclusion of vegetable ingredients, leading to competition with human food resources. Recent times have seen growing emphasis on sustainable feed ingredients. The Aquafly project led by NIFES (the National Institute of Nutrition and Seafood Research, Norway) is exploring the commercial feasibility of rearing Black Soldier Fly and kelp fly on a marine macroalgae supplement, and feeding the larvae to sustainably farmed Atlantic salmon. Both stranded and cultivated seaweed represent an abundant organic material which would provide a source of Omega-3 for the insect diets and therefore the marine fish diets. However, mass production of seaweed-fed insects as feed for fish in the EU is currently limited. This is due in part to major knowledge gaps relating to potential pathogen contamination acquired from the environment, and whether these pathogens pose a risk to both fish and, ultimately, to human consumers. The research objectives were to identify the extent to which the level of seaweed contamination by bacterial pathogens is determined by seaweed species and the environment from which the marine macroalgae are sourced, and to understand how the methods used to process the seaweed into insect feed affect pathogen survival and transfer throughout the production chain. Aquafly conducted a feed trial involving five seaweed species (*Laminaria digitata*, *Fucus serratus*, *Ascophyllum nodosum*, *Palmaria palmata* and *Ulva lactuca*) and utilising the facilities and expertise of commercial partners. Screening for environmental bacteria (coliforms and Enterococci spp.) was undertaken at a seaweed harvesting factory in Ireland. Samples were taken at every point in the production process, from the fresh seaweed and surrounding seawater, to the production of dried seaweed powder, to determine both the presence and persistence of bacteria. The seaweed powder was included as a supplement in the feed of Black Soldier Fly (BSF) larvae at a rearing company in the Netherlands. Samples of the powder and other raw feed ingredients, adult flies, post- harvest larvae and their frass, and the final processed larvae meal, lipids and chitin were all screened for environmental pathogens. The larvae meal was processed into fish feed pellets and fed to farmed Atlantic salmon in a seawater feeding trial in Norway. Microbiological sampling of pellets was undertaken during their manufacture and again prior to the start of the feed trial. This study provided an indication of potential sources of environmental bacteria into a candidate feed substrate for insect production,

and assessed the microbiological risk associated with feeding Atlantic salmon BSF larvae reared on a seaweed supplement. It also generated important data regarding persistence and die- off of bacteria in seaweed, BSF larvae and feed pellets during industrial processing. This research contributes to the wider conversation about exploiting waste organic materials as feed substrate for insect farming, and specifically addresses feed safety concerns and the risk posed to human consumers at the end of a novel, sustainable feed and food chain.

Keywords: Seaweed, Black Soldier Flies, aquafeed, environmental bacteria, human health

More sustainable food design with insects, agri-food waste streams and high-moisture extrusion

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A rapid increase of world population and a lack of traditional protein sources create preconditions for the search of alternatives and development of new acceptable food products. Insects currently are perceived as an alternative source of proteins. However, their level of impact as well as costs of production and processing is not competitive with the vegetable proteins (e.g. soya). We argue that the application of insect biomass grown on selected agri-food waste streams (DDGS, mill bran) in combination with twin screw high-moisture extrusion for the processing can provide more sustainable meat substitutes with similar to meat texture. Application of underutilized agri-food waste streams lowers the environmental impact and costs of insect biomass production.

Agri-food waste is a major problem of food supply chains efficiency and sustainability. Food supply chains are the biggest cause for greenhouse gases (GHG) emissions, water depletion, land use and biodiversity loss. Non-efficient resources use and high amounts of wastes generated along the supply chain are the main reasons for the unsustainable character of existing agri-food supply chains. In order to deal with 50% of diverse agri-food waste biomass generated along the supply chains a number of interlinked solutions is required. The solutions should be based on available knowledge, introduction of innovative solutions and technologies, reduction and prevention of food waste, improvement of food system governance and sustainable diets promotion. EntoWaste project (ERA-NET LAC) introduces a combination of solutions based on the use of insect organisms for agri-food waste transformation and valorization in EU and LAC.

EntoWaste project deals with a three-fold innovation through (1) the development of a modular waste utilization technology, (2) insect-based feed trials for the most common and regionally important animals, and (3) consumer-oriented food product development and design. Depending on the location the application of EntoWaste aims at multiple outcomes. The reduction of food waste (up to 50 M tonnes in EU and up to 70 M tonnes in LAC), creation of protein additives for EU pet food market of 9 M tonnes with turnover of € 15 billion (FEDIAF, 2014), generation of alternative protein sources for feed and food are the most promising areas for insect technologies. Previous results from Entomofood (Cornet project) demonstrated that of mixture of protein concentrates (insect with concentration of 15-50% in dry matter and soy) and water resulted in fibrous meat analogs with texture and protein composition similar to meat. Further studies indicated that extrusion of fresh insects (larvae of *Tenebrio molitor* and *Alphitobius diaperinus*) in combination with soya protein concentrate and water eliminated the microbiological ac-

tivity of insect biomass, further reduced the cost and environmental impact of meat substitutes production. The application of fresh insects directly for the extrusion processing allowed to produce meat analogs with similar protein and fat content excluding the need for expensive drying, freezing, milling and fat extraction. Moreover, it excluded the need to deal with the generated by-product (fat). Currently, insect fat has a limited application in food or feed industries and considered in many cases as a waste.

Therefore, more sustainable production of food based on insect biomass can be assured with the application of selected agri-food waste and side streams and whole fresh insect processing with high-moisture extrusion which assures the microbiological safety and similar to meat properties of extrudates.

Developing a breeding method for the nutritionally important edible shea caterpillar

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Shea caterpillars, *Cirina butyrospermi* are harvested and sold as food across West Africa, they are a significant source of income for rural women, and a valued protein and micronutrient -rich animal food. Increasing access to the caterpillars as a food source can improve the nutritional status of the rural communities in West Africa. Although abundant *C.butryospermi* is seasonal and a specialist feeder, eating only the leaves of *Vitellaria paradoxa*, the shea tree, making it difficult to breed domestically (ADEPOJU & DABOH 2013, ANDE 2003). No previously documented attempts have been made to breed *C. butryospermi* in a captive setting but related species have been successfully bred. We present the results of trials to establish a domestic breeding protocol. Wild *C. butryospermi* pupae were collected in Burkina Faso and sent to the UK for trials to break the diapause. Seven artificial diets were tested at two lab sites, one in the UK and one in Burkina Faso; six artificial diets were tested in a rural village in Burkina Faso. Fresh shea leaves were utilized as the control diet. Size, weight, and feed consumption were measured. Trials demonstrated that pupae can be successfully triggered out of diapause in a climate controlled cabinet set at 34°C with 75% humidity, 12hour day/night and the periodic introduction of 'rain' via a watering can. Artificial feeding trials are on-going and results will be forthcoming. This research offers positive signs that *C. butryospermi* can be breed in a domestic setting. Continued steps to establish a protocol for breeding *C. butryospermi* will significantly increase access to an important source of nutrition and income for rural communities. Future work will aim to refine the artificial diets and replace presently utilised lab ingredients with easily-sourced local ingredients, allowing the feed to be made at a much lower cost to the community.

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The trigger BSF eggs hatching and how to employ it in an large scale facility

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Millibeter is an insect company founded in 2012 breeding the Black Soldier Fly (Hermetia illucens) to bioconvert organic side streams into chitin, lipids and proteins. We are currently upscaling our R&D facility to a full-scale commercial production unit. In this process we have been able to refine well-known breeding practices as well as develop new techniques. One such technique is the larvae counter, a mechanism allowing us to count newly hatched larvae with a 97% accuracy.

The eggs are kept over a conveyor belt and the larvae are counted just before they roll off. This set up made it possible to keep track of the diurnal rhythm of the egg hatching. As the eggs are ovipositioned throughout the day, we expected the larvae to hatch somewhat continually spread over 24h. Nevertheless we observed very high peaks in larvae hatching alternated by lulls in productivity. These peaks and troths present a challenge to the scaling process, as it compromises the continuity of the system.

In an industrial setting, the high number of bins needing to be filled with larvae on a daily basis require handling processes that can be spread over at least 12h. Although our results posed a challenge to the industrial upscaling, finding the trigger of the larval hatching helps steer the process in time and location to fit the industrial schedule.

Here we will explain how we determined the trigger of this synchronized the hatching process and how we used the larval hatching trigger to manipulate and fit it to our schedule.

Insects for food: protein fractionation

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Insects are now seriously considered as an alternative and additional source of protein in developed countries in view of an increasing world population and the environmental problems caused by conventional cattle. To overcome the bias of people in the western world against insects as food, especially when the insects are offered in a recognizable form (including egg, larvae, pupae or adult), insect grinding /protein extraction is an option. The aim was to identify insect proteins using LC-MS/MS and to investigate protein digestibility (*in vitro*) of the whole *Tenebrio molitor* and its fractions (supernatant, pellet and residue) obtained using an aqueous extraction procedure. Proteins were more digested after pepsin/pancreatin digestion than after only pepsin digestion. The digestibility (estimated using the OPA method) of the supernatant fraction (~80%) was much higher than that of pellet fraction (~50%) and residue fraction (~24%) after *in vitro* gastro-duodenal digestion. Furthermore, the protein content of defatted pellet and residue (~80%) was higher than that of supernatant (~57%). The most abundant proteins were hemolymph protein (~ 12 kDa) & putative allergens (e.g. arginine kinase ~30 kDa) in supernatant fraction, and mainly muscle proteins (e.g. actin 30 - 50 kDa) in the pellet fraction. We further investigated how pH and NaCl affected protein yield of water-soluble protein fractions of *T. molitor* as target protein source, while preventing brown colour formation during protein extraction in order to obtain high protein quality. Minimum solubility was found at pH 4 - 6 with a recovery of 29.6% and maximum solubility was found at pH 11 with a recovery of 68.6%. Furthermore, extracting protein at 0.1 M NaCl, pH 10 gave the highest recovery up to 100% (Dumas analysis). After acid precipitation at pH 4, an isolate with a protein content of 74% was obtained. This isolate contained 22% of total protein present in *T. molitor*.

Insect protein as a food additive

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Entomophagy in developed countries often evokes fear, dislike and disgust reactions from consumers, but with the increasing global population, consumer demand for protein, dairy production costs and trends in dairy free, there is an increasing demand to look for alternative sources of protein to deliver food microstructures desired by consumers, insects of course being one alternative.

Several published consumer studies (1-4) have concluded that the inclusion of “invisible insects” as a powder or extract in food products could pave the way to enhancing consumer acceptance to a level where the consumption of unprocessed insects may be agreeable.

Based on these findings, we have investigated the utilisation of “invisible insects” as a protein ingredient to provide structure to food products. Proteins are essential constituents of food products not only for their nutritive value but for their functional properties. The functional properties of the protein ingredients allows the stabilisation of microstructures and indeed dictates the textures of salad dressings, spreads, bread, snacks, meringues, ice cream, cakes and milk froth.

In our contribution, we will present on the functional properties of a protein extracted from mealworm larvae which have allowed the creation and stabilisation of food emulsions, foams and gels.

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High moisture extrusion of two types of insect protein (*Tenebrio molitor* and *Alphitobus diaperinus*) with soy protein concentrate: influence of insect content and barrel temperature

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Meat production and consumption are responsible for the highest environmental impact in the food sector. In order to provide more environmentally friendly alternatives, a number of meat substitutes was developed and faced the market with different success rate. Insects became a viable alternative of food proteins in Western countries quite recently. Interest in insects is progressing due to their high feed to proteins conversion rate, their high protein and/or fat content, their potentially low environmental impact and low land use for production. Further progression of insects as alternative protein source for food depends in a great degree on the assurance of their safety and nutritional quality. Moreover, it is recognized that the similarity in taste and physical properties (e.g. texture) of new products (meat substitutes) plays an important role in acceptance of alternative foods. That is why the creation of an insect substitute, which imitates meat texture in its full complexity, is a great challenge both in a scientific and technological way. This study aimed at the design of high-moisture extruded intermediates (HMI) based on insect biomass, having texturized properties similar to the most successful meat substitutes (HMI based on soy). The use of insect biomass instead of soy could improve the protein and amino acid profile of the substitute and make it animal derived. Moreover, its inclusion should encourage the reduction of the European dependency on external soy supplies. Specific objectives in this paper include testing of different extrusion parameters, such as feed compositions, water pumping and cooking temperature settings. The obtained HMI-products were then tested for protein and water content and protein solubility and they were characterized by texture profile analysis and scanning microscopic images of the texture. Soy protein concentrate (protein 69%; carbohydrate 12-18%) as well as soy fiber were obtained from Solae LLC (St. Louis, USA). *Alphitobius diaperinus* protein concentrate (68% protein content DM) was purchased from Proti-Farm Holding NV (Ermelo, The Netherlands), while *Tenebrio molitor* protein concentrate (66% protein content DM) was acquired from HiProMine (Robakowo, Poland). High-Moisture Extrusion was performed in DIL (Quakenbrueck, Germany) on a co-rotating twin-screw extruder (model Berstorff ZE 25x33D). The adversity and hardness of high-moisture extruded samples were determined using a TA-XT2i texture analyser (TA Instrument, Vienna Court Stable Micro Systems Ltd., United Kingdom). The analysis of the extruded intermediates demonstrated that they have a great variation of cutting

strength results, which dependent on sample composition and extrusion parameters. Increasing the concentration of insect protein powder in the samples with similar other conditions (temperature 170°C, water setting 45) decreased the cutting strength in the samples. At the same time the change of processing parameters (temperature 160°C and/or water setting 40) resulted in improved hardness of the samples. Increased water pumping in the samples (water setting 45) resulted in increased protein solubility, which in turn resulted in formation of a less “firm” texture and low cutting strength. Inclusion of soy fiber did not increase the hardness of the samples with water setting 45. Inclusion of soy fiber (5 or 10%) in samples and maintaining the water content at setting 40 increased the cutting strength and hence hardness to reach values similar to chicken breast meat (initial hardness and shear force ranging 5.8-13.8 N). Cutting properties of insect-based meat substitutes did not reach values similar to those for pork and beef. Scanning electron microscopy (SEM) analysis of the samples indicated that the increase of insect biomass in the extruded samples diminished the micro-texture of the samples. While the samples with 15 and 30% (on dry matter basis) of insect protein powder still had a recognizable porous, fine and directional texture (similar to pure soy concentrate standards), samples with higher concentrations (50-70%) were characterized with a more bulky structure. Samples with 40% of insect biomass had more flat layered texture (not as fine and porous as a standard). Addition of soy fiber (5% and 10%) made their texture more directional and finer (protein content 25.0-30.8% on a wet basis). Similarly to cutting strength results, SEM indicated that decreasing the water input (at the same temperature and composition) resulted in a more textured structure of the products. Most probably such results are related to lower extrusion moisture causing an increase in shear and friction inside the cooling die, resulting in a better texture, greater velocity gradient of the material in the die and fiber formation. The research demonstrated that high-moisture extrusion can induce the formation of a meat-like fiber texture in meat substitutes consisting of a mixture of protein concentrates (soy and *Alphitobius diaperinus* or *Tenebrio molitor*) and water. Sample composition and water content were the main factors influencing the cutting strength of the high-moisture extruded intermediates. It was possible to preserve the meat-like texture adding up to 40% of insect protein concentrate in a mixture with 5-10% soy fiber (dry matter), which resulted in meat analogs with 25.0-30.8% of protein content. Other factors like amino acid losses, protein-protein interactions or the formation of peptide bonds in extruded samples were not a part of this study.

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Keywords: Lesser mealworm (*Alphitobius diaperinus*), yellow mealworm (*Tenebrio molitor*), meat substitute, alternative protein source

Insects as ingredients for bakery goods. A comparison study of *H. illucens*, *A. domestica* and *T. molitor* flours

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The search of new alternative sources of proteins have prompted the food industry to look at the insects as a potential raw ingredient. Insects are becoming an interesting source of proteins for feeding, but less attention has been paid as a protein-rich food source for humans, which must be considered due to the growing world population. Insects might be an ecological and healthy commodity that could contribute to global food security, taking into account the high biological value of their proteins. Furthermore, comparing with conventional livestock, insects need less feed and water, and they release less greenhouse gases, consequently they are a more sustainable source of animal proteins. Owing to their appearance, the ingestion of raw insects is not well accepted due to cultural traditions. Therefore, their incorporation in food products as flour could provide a good alternative for masking their presence whenever required. One of the election products for the inclusion of insects as a powder would be the bakery goods, namely bread, because it is known worldwide and even considered staple food in some countries. The aim of this research was to explore the potential use of insects' flour as protein-rich ingredient for bakery products. For that purpose, three insects from different Families were selected, using larvae or adults depending on the order. *Hermetia illuscens* (Diptera, Black Soldier Fly larvae), *Acheta domestica* (Orthoptera, crickets) and *Tenebrio molitor* (Coleoptera, yellow mealworm) were the insects selected. Proximate composition of insects flours was determined including proteins, fats, minerals and nitrogen free material. Insects flours (5%, w/w f.b.) were blended with wheat flour and the composite flours were subjected to breadmaking, previous rheological characterization of the resulting doughs by using the Mixolab. Breads were characterized regarding technological properties and nutritional composition. As expected, insect flours had higher content of proteins than wheat flour, being the highest value obtained with *A. domestica*. Likewise, insects showed higher content of fat and minerals, with very low amount of non-nitrogen material. The inclusion of insects' flour affected the rheological properties of dough during mixing, requiring lower water adsorption and hydration. All insects' flours increased the stability of the dough during mixing. Breadmaking process could be carried out with all the composite flours leading to breads with different technological characteristics. Breads containing *Hermetia illuscens* flour showed the darkest crumbs and a compact crumb texture. All breads enriched with insects' flours showed high content in proteins and fat than the wheat bread. Overall, results confirm the usefulness of insects' flour for protein enrichment of bread, although a reduction in the fat content would be advisable for better nutritional balance of breads.

Evaluation of the chemical safety of edible insects and insect-based food intended for human consumption. A Belgian pilot study

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Due to the rapid increase in world population, the use of alternative and more environmentally sustainable food sources is strongly encouraged. In this perspective, the opportunity for insects to represent a valuable alternative to main animal food sources (e.g., meat and fish) is remarkable. However, despite their high nutritional properties and more environmentally sustainable production, edible insects are generally perceived as an unappealing food source, especially among Western countries. The chemical safety of edible insects can thus contribute to the process of acceptance of insects as an alternative food source. In response to the recommendations expressed in a recent scientific opinion adopted by EFSA (2015) on the "risk profile related to production and consumption of insects as food and feed", the present study aimed to provide a comprehensive overview of the residual levels of different chemical compounds (including brominated and phosphorous flame retardants - BFRs, PFRs; polychlorinated biphenyls - PCBs; organochlorine compounds – OCPs; dioxins and dioxin-like PCBs; pesticides; metals - As, Cd, Co, Cr, Cu, Ni, Pb, Sn, Zn) in four species of edible insects (*Galleria mellonella, Locusta migratoria, Tenebrio molitor, Alphitobius diaperinus*) and four insect-based food items currently commercialized in Belgium. Our results pointed out a general low chemical contamination of the analyzed samples. In particular, the levels of PCBs ranged from 26.5 pg/g ww to 2,065 pg/g ww, with an average mass fraction of 743 (±745, SD) pg/g ww. Among the various OCPs examined, HCB, DDT and HCHs were detected in almost all the analyzed samples, with total levels ranging between 46.3 and 368 pg/g ww. Total BFR levels were generally < LOQ and never exceeded 35.5 pg/g ww, while the total levels of PFRs were generally higher (from 783 to 23,786 pg/g ww). The total levels of dioxin compounds measured in the analyzed edible insects ranged from 0.05 to 0.28 pg WHO-TEQ/g ww. Cu and Zn were consistently the most abundant metals, with mass fractions ranging from 0.85 to 9.12 mg/kg ww and from 6.44 to 58.60 mg/kg ww, respectively. Lower levels were measured for Co (<0.05 mg/kg ww), Cd (<0.06 mg/kg ww), Cr (<0.24 mg/kg ww), and Ni (<0.28 mg/kg ww), while As, Pb, and Sn were consistently < LOQ. The pesticide suspect-screening revealed the presence of vinyltoluene, tributylphosphate, pentafluoropropionic acid, and some pesticides (e.g. methoprene, empenthrine, pirimiphos-methyl), but it was not possible to identify a clear contamination pattern between the insects and the insect-based food. In addition, the chemical levels measured in edible insects were compared with

those found in other studies reporting contamination levels in meat, fish and seafood, and eggs at levels considered safe for human consumption. Overall, our results support the possibility for humans to consume these insect species with no additional hazards in comparison with commonly eaten animal products, and indicate that the analyzed insect food could be considered a valuable alternative to common sources of proteins.

The dynamics of inheritable cultural gastronomy in relation to commercial food product-positioning within Europe

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To make novel food products a commercial success, they have to be positioned in the right way. In Europe, different nationalities inherited different gastronomic cultures. Choosing a different positioning strategy based on these differences leads to different adoption behaviour of novel food products.

When it comes to the insights on positioning of food products adjusted to regions the following events were important: in 1983 there was Levitt's influential statement "think global, act local", in 1998 Askegaard's work on differences at national and regional level in food-related behaviour was published and in 2006 Trichopoulou confirmed this statement by saying that traditional foods still left an imprint on the contemporary diet. However, through globalization the world is becoming increasingly uniform; technological advancement has increased global accessibility, product transportation has become easier, and the internet enables people to individually access an enormous amount of information and to get in touch with others fairly easily.

In order to examine whether this still is the case in 2017 within Europe, a multi-lingual survey was carried out within five European countries (The Netherlands, Germany, France, Italy and Spain) primarily by the use of social media. In this survey an exquisite experience for the respondent was the focus of attention. To compare the obtained data and to see if there were differences between nationalities the data was analysed in SPSS using ANOVA, MANOVA and RM-MANOVA tests.

The results show that there are significant differences between European nationalities when it comes to novel food acceptance. The proposed conceptual framework provides the key factors that influence novel food adoption behaviour.

Keywords: novel foods, edible insects, edible algae, positioning, food neophobia, food choice motives, European consumers behaviour, gastronomic heritage, influence of information on consumer behaviour, influence of knowledge on consumer behaviour, consumer adoption behaviour.

Effects of gender and rearing substrate on protein profile of silkworm (*Bombyx mori*) pupae

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The consumption of insects, or entomophagy, is traditionally practiced in many parts of the world (tropical and subtropical countries in Africa, Asia and Latin-America) where more than 2000 edible insect species are collected in the wild up to today. Entomophagy could contributes positively to the environment, health and livelihoods. The environmental benefits of rearing insects for food are mainly founded on the better feed conversion efficiency of insects respect to those of chicken, pork and beef (VAN HUIS 2013; JONGEMA 2014). In addition, insects can be reared on organic side-streams and are reported to emit lower amounts of greenhouse gases and ammonia, requiring significantly less land and water (FAO 2013). Generally, they were found to be highly nutritious and to represent good sources of proteins, fat, minerals, vitamins, and energy compared to other animal foods (FAO 2013).

On the other hand, in spite of the nutritional interest, the food safety of edible insects has not been extensively studied but due to the increasing interest of insects as new protein source in the last few years, the European Commission asked the European Food Safety Authority (EFSA) to assess safety risks arising from production and consumption of insects as food and feed. On October 2015 EFSA provided an overall conclusion where reported that a specific risk assessment should be performed taking into account the whole production chain from farming to consumption, including the species to raise and the substrate to use as well as the methods for farming and processing (EFSA 2015).

Among edible insects, the silkworm (*Bombyx mori*) pupae (SWP), which are massively produced as by-products of the sericulture industry, can be suitable candidates because they could be reused as a protein source. SW has been included in the EFSA list of insect species with the biggest potential to be used as food and feed in the EU even if a specific risk assessment should be still performed, including the evaluation of the allergenic potential associated to entomophagy (EFSA 2015).

The aim of this work was to characterize the protein profile of SWP by 1D and 2D-PAGE coupled to mass spectrometry (MS), in order to identify the most abundant spots (relevant from the nutritional point of view) and any gender and/or rearing substrate dependent differences in protein expression. Three different batches of SWP from each gender reared on natural (mulberry leaves) or an artificial patented diet (CAPPELLOZZA *et al.* 2005) were analyzed. Protein extracts were separated by 2D-PAGE (3 technical replicates for each sample), followed by image and statistical analyses (Shapiro Wilks fol-

lowed by a one-way analysis of variance and Tukey test with p≤0.05). Both most abundant and gender/rearing-dependent differentially expressed proteins were excised to be identified by MS analysis.

Around 60 spots were differentially expressed in at least one of the experimental conditions, considering a 3-fold variation threshold. Among these proteins under identification, major proteins are typical SWP's hemolymph proteins, among these differentially expressed ones are gender-specific (e.g. vitellogenin). Another protein included in the most abundant sub-class is the Lipoprotein 11 already known as food allergen (ZHANG *et al.* 2006). This complex proteomic study could improve the state-of-the-art knowledge about edible insects in term of protein content and protein characterization, both for nutritional and safety evaluation purposes.

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Industrial rearing optimization of *T. molitor* larvae (Coleoptera, Tenebrionidae) with respect to high rearing densities, yeast as food additive and alternative possibilities of non-invasive monitoring systems

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Pest insects like *Tenebrio molitor* L. (Coleoptera, Tenebrionidae) are more and more used to produce high-quality protein. Optimisation and automatization of mass rearing practices of insects are being developed and analysed to satisfy these emerging demands. Under the pretext of mass rearing insects are raised under extremely high densities. In order to examine the correlations of high larval density on food utilization and biomass accumulation by *T. molitor* larvae, we performed tests to measure the efficiency of digested food conversion (EDC), ingested food conversion (ECI) and gained weight of larvae biomass per food consumed at increasing larval densities (44, 351, 547, 732, 914, 1097 larvae per dm²) over three consecutive 3-week periods. Subsequently to appropriate rearing conditions, mass rearing as well as further processing of insects requires the realisation of suitable and controllable approaches. In this context, establishing and integrating non-invasive process monitoring systems is an essential prerequisite for the implementation of reliable process control strategies. During breeding non-invasive measurement methods using camera measuring techniques and image processing algorithms, enable the real-time process monitoring by detecting pupae or even mealworm beetles e.g. in a breeding box. The detection of pupae facilitates the determination of an adequate time frame for reaping the insects, so that a machine vision-selection-system could be used to maintain the breeding of following mealworm generations. Related systems have already been established to remove for example grains with defects. The classification algorithm uses shadows, edges and colours to detect these damaged grains (PEARSON *et al.* 2012). As unhighlighted pupae in a box are hard to detect, the adaptation and application of concatenated histograms of local appearance features could serve as a method to detect pupae in a full box (LARIOS *et al.* 2008). The presented research adapts and applies these techniques for the rearing of insects in order to realise more efficient sorting and reaping processes to serve as a basis for the establishment of a robust and high-grade industrial rearing. Hence, we performed experiments with a monochrome smart-camera (Pictor T303-M-ETH, Vision and Control GmbH) in a lab scale rearing system. Single-sorting algorithms were tested with different larval densities on the image section, as well as pattern recognition algorithms with clustered larvae. In order to additionally detect protein or fat composition of the larvae and the pupae, NIR-spectroscopic measurements are considered as supplement of

the monitoring system. These parameters could contribute as further criteria for refining the sorting unit.

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Preliminary results of impact of ph in diets on the growth of black soldier fly (*Hermetia illucens*, L) larvae

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Diet parameters influence the development of black soldier fly larvae. An understanding of the various factors having such an impact is critical as diets can be manipulated to maximize production systems with the black soldier fly (BSF – *Hermetia illucens* L.). As is well-establish, the BSF is a recognized method for waste management (NGUYEN *et al.* 2013) for production of proteins, fat, and other products, for use as animal feed (HENRY *et al.* 2015, GASCO *et al.* 2016, SCHIAVONE *et al.* 2017). Previous research examined the effect of medium type on BSF (TOMBERLIN *et al.* 2002, TSCHIRNER & SIMON 2015). The roles of abiotic factors were studied previously by HOLMES in 2010, but this study only considered the effect of humidity and temperature on BSF development. The ph effect on larvae was considered only by two previously studies (POPA & GREEN 2012, BANKS 2014). From these studies, pH significantly impacted larvae; however, the larvae can change it during their growth. BSF larvae elevate the pH of liquid solution (leachate), as detected by POPA & GREEN (2012), from 4.0 to 9.0 in 7 days. ALATTAR (2012) determined pH ranged from 8 to 9 after 8 days of activity but the rate at which pH shifted depended on larval density: with high densities this process was accelerated. The aims of the current research were to evaluate i) pH diets effects on the growth (time and weight) of BSF larvae and ii) how two system feeding impacted these patterns.

A trial was performed to evaluate three different level of pH in diet (4.0, 7.5 and 9.5) with one control diet (6.1) included. The Gainesville diet, 11g/per day dry matter (70% moisture) was used based on previous work by TOMBERLIN *et al.* (2002). The pH was adjusted using a 0.1 M solution of sodium hydroxide and 0.1 M solution of citric acid. The pH of the control was not altered. Four replicate per treatment were performed using 500 larvae (5 days-old) per replicate. Each group of larvae was allocated in a plastic container (32x20x7cm). The containers were placed in a climatic chamber with temperature (28°C±0.5) and relative humidity (70±5%) controlled and under 14:10 light/night photoperiod. Two different feeding systems were employed. All amount of diet was dispensed from the beginning of each ph treatment in the first feeding system (all feeding system AFS) while 40g/day of diet was distributed in the second system feeding (daily feeding system DFS).

Every day, ten larvae per replicate were randomly selected and individually weighed with a scale (0.0001 g) and reintegrated to estimate the larvae's growth rate at the same

hour (9:00 a.m.). A sample of diet was collected in each replicate to monitor daily pH variation rate (Hanna Instrument HI5221; Woonsocket, RI 02895 USA) and reintegrated in each container. The trial was stopped when 40% of larvae reached the prepupae stadium, dark brown sides as described in MAY (1961). Each larvae treatment container was separated and 10 larvae per replicate were weighed to evaluate the final weight.

The weight and pH variation were subjected to two ways-ANOVA-repeated measurements (fixed effect: treatment and feeding system) to evaluate effect of different pH level and feeding system on the larvae's growth rate, significance was fixed p-value: <0.05. No statistical analysis was performed when the model was not balanced.

The growth rate of BSF and pH variation during the trial is showed in **Figures 1**. No significant differences were showed between different pH diets during the trial. Feeding systems showed significance differences (p-value<0.000) during the trial. The final weight showed significance differences between the two feeding systems (p-value<0.033). **Figure 2** reports the variation of pH operated by BSF in the two feeding systems.

The pH showed high differences in the first days of trial (1-3 and 4-5) (p-value<0.000), while no differences were highlited from 6-9 days no differences. BSF modified pH in all treatments and stabilized it around pH=9 at the end of the trial. The pH diet seems not directly influence the weight of the larvae but it could affect the growing time. As reported, the DSF influence larvae' growth rate and they reached the prepupae stadium two days later if compared to AFD. The feeding system seems be the real impact on the growing time of larvae because the larvae in DFS spend a part of their energy to change the pH every day. The pH variation ranged between 8.5-9.2 like showed in POPA & GREEN (2012), it seems maintained constant by larvae's activity, in the two feeding system too. "The system do not allow to insert figure"

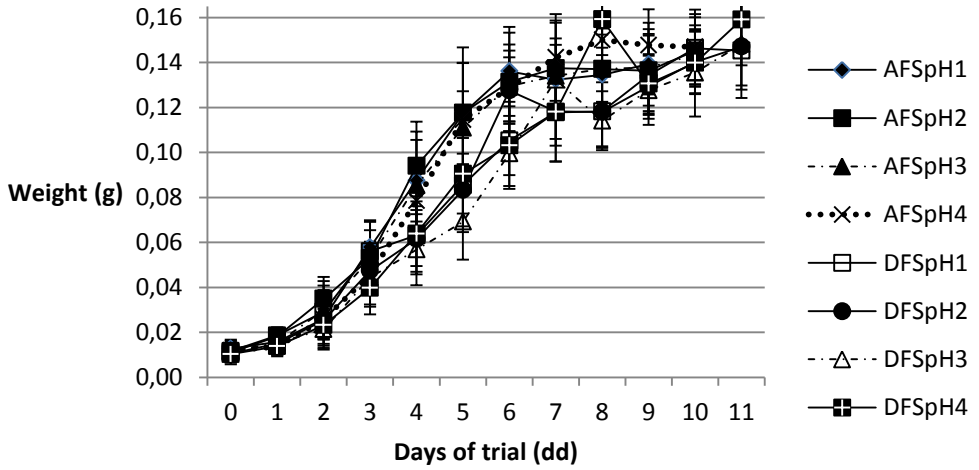

Figure 1: Growth rate of BSF fly reared with three level of pH and one control diet, using two different feeding system: all amount of feed distributed from the beginning (AFS) and daily feeding system (DFS). pH1: low basic (7.5); pH2:control (6.1); pH3: high basic (9.5); pH4: acidic (4.0) AFS: all amount feeding system, ADS: daily feeding system

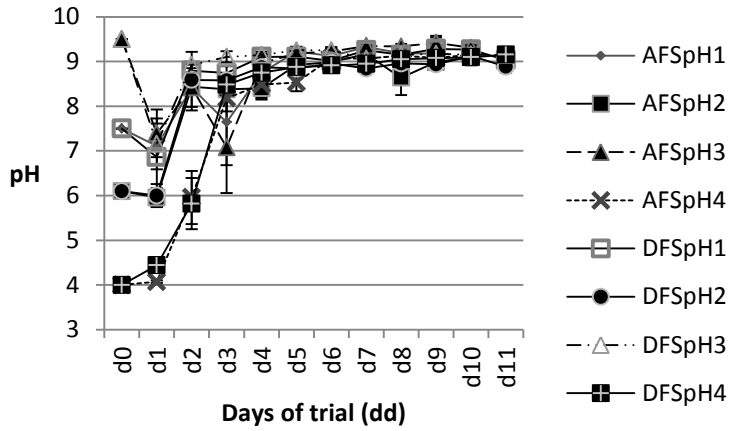

Figure 2: Variation of pH operated by BSF in the two feeding systems., pH1: low basic (7.5); pH2: control (6.1); pH3: high basic (9.5); pH4: acidic (4.0) AFS: all amount feeding system, ADS: daily feeding system

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How to manipulate fatty acid contents of edible insects using artificial diets

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Dietary manipulation of fatty acids in edible insects could offer a solution for the growing need to find more sustainable sources of essential fatty acids for human nutrition. Currently the recommended ratio of omega-6 and omega-3 fatty acids is 5:1 but in the Western diets, the intake of omega-6 is too high. Some insects are able to store dietary fatty acids largely unaltered to their fat body and thus the fatty acid content could potentially be designed with modified feed offering a new source of omega-3 fats. Edible grasshopper, *Ruspolia differens*, is one of the most consumed insects in Africa and extremely rich in fat. We asked how artificial feeds with manipulated content of essential fatty acids influence the nutritional composition of *R. differens* and its performance. We reared individuals in laboratory with seven artificial feeds where essential fatty acid, saturated fatty acid, carbohydrate or protein levels had been maximized. The seventh diet treatment was a mix of the six diets. We were able to drastically modify the fatty acid composition and concentrations of the insect. Lack of protein and fats in the diet prolong the development and leads to lower weight. Our results also suggest that omega-6/omega-3 –ratio of *R. differens* can be lowered below the recommended ratio with diet. These findings offer new sustainable possibility to improve human nutrition by producing edible insects with designed nutritional content.

The residual biomass landscape for insect production

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Topic: Evaluation of the use of insects; Status of legislation

The global demand for food is growing, which poses challenges for the limited resources we have available for food production on the planet. In particular the rapidly increasing demand for poultry and pork meat and fish is challenging as the required protein sources used in animal feed, e.g. soybeans and pelagic fish, are already associated with over-exploitation of land and ocean resources. To keep pace with these demands, we urgently need innovations that increase both the productivity and the efficiency of our food system. Insects can play a key role as they have the potential to convert low-valuable biomass sources, now lost in the food production system, into high-quality and sustainable proteins for animal feed. The benefits of this concept, however, largely depend on the characteristics of the biomass sources used to feed insects. The aim of this project was therefore to provide a comprehensive overview of available low-grade biomass sources in the food production system and their potential to grow environmentally friendly, economical, and safe insects. The approach involved a legal and safety analysis; a review of availability and suitability; and a study on the environmental impact of potential insect substrates. This approach and the preliminary results of this ongoing project will be presented.

IPIFF position paper - Implementation of EU Regulation 2015/2283 on 'novel foods'

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On 25 November 2015, the EU legislator adopted Regulation (EU) 2015/2283 on 'novel foods': the regulation introduces a new procedure for authorising 'Novel Foods' for commercialisation on the EU market. Replacing the current EU Regulation (EC) 258/97- this new text will apply as from 1st January 2018.

The EU legislator clarified the legal status of insects and their derived products – including for 'whole insects & their preparations' (see recital 8 of the text) - which are now explicitly covered under the new Novel Foods (NF) legislation: consequently, insect products which have not been 'consumed to a significant degree within the European Union (EU) before 15 May 1997' must be assessed and receive a European authorisation with the view to be legally placed on the EU market as from 2018.

IPIFF considers that the establishment of harmonized rules for the marketing of insect products is a 'step in the right direction': while insects have the potential to become a major source of protein in Europe, insect producers primarily rely on a 'solid' & 'stable' EU regulatory framework to plan their investment & marketing activities throughout the continent.

IPIFF emphasises, however, the importance for EU authorities to establish 'workable' rules & to provide guidance at implementation stage. These principles, along with appropriate transitional measures, are notably relevant to facilitate the uptake of this new legislation by the insect producers.

IPIFF position paper - The use insect proteins as animal feed

IPIFF believes that insects will soon constitute a reliable alternative or addition to fishmeal feed formulae for aquaculture: insect nutritional characteristics (e.g. protein content, amino acid profile and/or digestibility levels) are indeed comparable to those of fishmeal products, making them a pertinent substitute or addition in the diet of certain fish species (e.g. trout or Atlantic Salmon) or shellfish (e.g. shrimps). However, the EU 'feed ban rules' contained in the so called 'TSE Regulation' (i.e. Article 7 and Annex IV of Regulation 999/2001) so far prohibits the use of animal derived protein to be used in feed for farmed animals, including for fish.

The European Commission recently published (on 15 November 2016) a draft amendment to the TSE Regulation (see above) which aims to partially uplift the feed ban regarding the use of insect processed animal proteins (PAPs) for aquaculture animals.

This draft amendment was voted & endorsed by a ‘qualified majority’ of EU Member States in December 2016.

- this draft text introduces a specific section for insects & insect products (Annex IV, section F of Regulation 999/2001) which allows insect-producers ‘to make use of the same authorisation’ as the one benefiting to those producing and processing other non-ruminant animals (i.e. pigs & poultry) for feeding aquaculture animals.
- The authorisation is however limited to seven insect species (see Chapter II of Annex X to Regulation 142/2011), namely to the followings: black soldier fly, house fly, yellow mealworm, lesser mealworm, house cricket, banded cricket & field cricket.
- Likewise other farmed animals, these species may only be fed with ‘feed grade materials’ (see below for more details). The same rules apply to insect PAPs that are imported from EU third countries.

The text is now expected to be officially adopted in May or June 2017 prior to its official application as from 1st July 2017.

Insect protein in aquafeed – potential of *Tenebrio molitor* meal as substitute for soy protein concentrate in compound feed for Tilapia (*Oreochromis niloticus*)

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In addition to fish meal (FM), soybean protein is the most common protein source used in compound aquafeed, but also associated with environmental and economic problems (1). Due to the rapid development of aquaculture the increasing demand for feed has initiated research about alternative and more sustainable proteins for aquafeed. Recent investigations suggest that insect meals could be an interesting option. In context of the expected upcoming approval as feed ingredient for aquafeed in 2017, the current study aimed to evaluate protein quality parameters due to graded substitution of soybean protein concentrate (SPC) by fullfat or partly defatted insect meal from larvae of yellow mealworm (*Tenebrio molitor*) in Tilapia diets.

Methods: A growth study was conducted with 400 juvenile all-male fishes of Nile tilapia (*O. niloticus*) using a control diet (7% FM, 31% SPC (Crude protein (CP): 74% of dry matter (DM)) and four experimental diets with 50 or 100% replacement of SPC by either fullfat (CP: 54% of DM, Crude lipids (CL): 37% of DM) or partly defatted (CP: 73% of DM, CL: 7% of DM) *Tenebrio molitor* meal (fullfat: TMff50/TMff100; partly defatted: TMdf50/TMdf100). All diets were formulated to be similar both in CP (39% of DM) and energy content (**Table 1**). Essential amino acid (AA) supply of the diets was within the recommendations for Tilapia. Growth response and protein utilization were studied in a semi-closed in-door water recirculation system with 20 tanks (320 l/tank; water temperature: 24.5 ± 0.3°C; regulated photoperiod: 14h light/10h dark). Four replicate tanks per diet (20 fish per tank) were utilized in a 56 d growth experiment by DM provision in two meals each at 2.2% body mass (BW). Ten fish at the beginning and 12 fish per diet at the end of the growth study were analyzed for body composition to generate N deposition data. Both parameters of growth response and protein quality were calculated according to (2, 3). Standardized net protein utilization (NPU_{std}) referred to an average of daily N intake ($NI=400mg/BWkg^{0.67}$) as observed. Statistical analyses (one-way ANOVA, Tukey-test) were conducted by R-software (version 3.0.2).

Table 1: Feed formulation

	Dietary inclusion level [%]				
	Control	TMff 50	TMff 100	TMdf 50	TMdf 100
Fish meal	7.1	7.1	7.1	7.1	7.1
Fish oil	6.5	3.7	0.9	6.0	5.5
Wheat meal	26.7	29.0	29.0	29.0	29.0
Wheat starch (native)	10.4	10.9	13.3	9.9	11.2
Wheat gluten	8.1	11.0	14.2	7.4	7.0
Tenebrio meal (partly defatted)	-	-	-	15.6	31.2
Tenebrio meal (fullfat)	-	15.6	31.2	-	-
Soy protein concentrate	31.2	15.6	-	15.6	-
Soybean oil	6.5	3.7	0.9	6.0	5.5
Vit./Min. Premix	1.0	1.0	1.0	1.0	1.0
$CaHPO_4$	0.7	0.7	0.7	0.7	0.7
$CaCO_3$	0.7	0.7	0.7	0.7	0.7
CMC (Binder)	1.0	1.0	1.0	1.0	1.0
Proximate composition [% DM]					
Dry matter content	89.4	89.5	90.2	90.1	91.1
Crude protein (N×6.25)	38.2	38.7	39.2	39.4	39.4
Crude lipids	15.9	15.7	15.0	15.9	16.3
Gross energy [MJ/kg DM]	22.1	22.2	22.2	22.3	22.4

Results: All diets were very well accepted. Replacement of SPC by *Tenebrio molitor* meal (TM) significantly improved protein quality (NPU_{std}) as well as specific growth rate (SGR) and feed conversion ratio (FCR). SGR and FCR were superior with diet TMdf100 but highest NPU_{std} was observed for diet TMff50 (**Table 2**). Body composition of fish was also affected by experimental diet (**Figure**). SPC replacement level up to 50% was most effective when fullfat TM was used but at higher inclusion level of TM the partly defatted variety becomes more beneficial. No supplementation of crystalline AA was needed to fulfil the current recommendations for Tilapia in each of the diets.

Table 2: Results of growth, feed efficiency and protein quality of experimental groups

Diet	Control	TMff50	TMff100	TMdf50	TMdf100
NPUstd [%]	47.8 ± 0.7 ^{a}	52.8 ± 0.5 ^{c}	49.8 ± 0.5 ^{b}	50.2 ± 0.7 ^{b}	50.8 ± 0.8 ^{b}
SGR [%]	4.3 ± 0.1 ^{a}	4.7 ± 0.1 ^{b}	4.8 ± 0.0 ^{b}	4.7 ± 0.1 ^{b}	5.0 ± 0.1 ^{c}
FCR [g/g]	1.29 ± 0.03 ^{a}	1.14 ± 0.02 ^{b}	1.12 ± 0.02 ^{b}	1.13 ± 0.02 ^{b}	1.05 ± 0.02 ^{c}

Means (± SD); SGR = specific growth rate; FCR = feed conversion ratio; NPU_{std} = standardized net protein utilization (standardized daily N intake = 400mg/$BWkg^{0.67}$); different superscript letters reveal significant differences between diets ($p<0.05$)

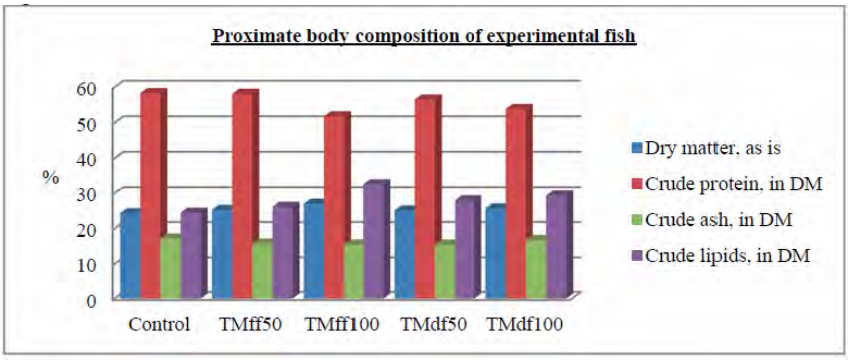

Figure: Effect of diet on proximate body composition of fish under study

Conclusion: Replacement of SPC by either fullfat or partly defatted *Tenebrio molitor* meal improved dietary protein quality of Tilapia diets under study and enhanced growth performance of Nile tilapia. Therefore, insect protein from *Tenebrio molitor* can be a further option to make aquafeed formulation more flexible and sustainable when approved as feed ingredient. Defattening of TM is recommended if dietary substitution of SPC exceeds 50% (equivalent to absolute dietary inclusion of 16% TM by weight). Ongoing research will examine if a further improvement of the dietary AA balance can be achieved with alternative protein sources in fish diets.

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Partial or total substitution of soybean oil by *Tenebrio molitor* oil or *Hermetia illucens* fat in rabbit diets. Preliminary results: effects on growth and slaughtering performances

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The 60-70% increase of animal products consumption expected by 2050 has pushed research to investigate new raw materials for animal feeds. In this context, insects are a very interesting and innovative feed for fish and terrestrial animals due to their valuable chemical composition and their claimed sustainability (VAN HUIS *et al.* 2015). So far, research has mainly been addressed to the protein content of insect larvae meals in fish, poultry and pigs feeds as conventional protein sources substitutes (BOVERA *et al.* 2016, GASCO *et al.* 2016) and the most insect species investigated were Black soldier fly (BSF - *Hermetia illucens*) and Yellow mealworm (TM - *Tenebrio molitor*). When processing larvae, and in order to increase the protein content and the storability of the meals, some authors underlined the importance of defatting larvae meal (HENRY *et al.* 2015). The extracted lipid could find uses as animal feeds (SCHIAVONE *et al.* 2017). Using lipid in the rabbit diets has an impact on growth and health of rabbits. In rabbit post-weaning stage, DJAKALIA *et al.* (2012) showed that the use of soybean oil (SBO) by its essential FA composition improves growth performance of rabbits compared to the use of the palm oil, which is rich in SFA. As far as rabbit are concerned, only two research have been performed using insect meal (silkworm) replacing soybean meal in diets for growing rabbits (CARREGAL & TAKAHASHI 1987, LIU *et al*. 1987) and no researches were ever been conducted using insect fats or oils. The aim of this research was therefore to assess the effect of BSF fat and TM oil used as partial or total substitute of soybean oil on growth performances and slaughter traits of growing rabbits.

Two hundred Hycol hybrids weaned rabbits (36 days old, initial mean body weight: 1050.71±137.85) were randomly divided into five groups of 40. Rabbit were fed ad libitum with a control diet (C) containing 1.5% of SBO tested against four experimental diets developed substituting the control diet SBO with 50 or 100% of BSF fat or TM oil (BSF50, BSF100 ,TM50 and TM100). The effects on growth performance were studied

from 36 to 77 days of age. During the experiment, live weight and feed intake were recorded per cage on a weekly basis. At the end of the trial, the means of live weight, average daily feed intake (ADFI), and average daily weight gain (ADG) were calculated. Feed conversion rate (FCR) was calculated as the ratio between the ADG and the ADFI.

At the end of the trial, 20 rabbits per group were slaughtered and weight recorded (SW). After 24-h of cooling, the commercial carcasses with head were dissected according to the procedures described by DABBOU *et al*. (2017). The chilled carcass weight (CCW) was recorded and the dressing out percentage was calculated as the ratio between CCW and SW. The reference carcass weight (RCW) was also calculated. The perirenal fat was recorded. The statistical analyses were performed using the SPSS software package (version 21 for Windows, SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to evaluate the effect of BSF fat and TM oil on growth performances and carcass characteristics. Mortality was calculated by Fisher's exact test and used control group as the reference. Significance was declared at $P<0.05$.

Table 1 reports the effects of experimental diets on growth performance. After 41 days of feeding trial, no difference was observed among the control and the groups containing insect oils in terms of performance and mortality.

Table 1: Growth performance of rabbits fed experimental diets (n=40 rabbits/group).

	C	BSF50	BSF100	TM50	TM100	P-value
Final live weight (g)	2906.75	2859.19	2879.57	2811.42	2917.30	0.544
ADFI (g)	154.18	149.82	153.23	149.29	153.55	0.553
ADG (g)	45.55	43.90	44.18	43.52	45.14	0.531
FCR	3.40	3.44	3.52	3.45	3.41	0.384

As far as slaughtering performances are concerned (**Table 2**), there were no differences among experimental groups for all the considered parameters ($P>0.05$).

Table 2: Slaughtering performance of rabbits fed experimental diets (n=20 rabbits/group).

	C	BSF50	BSF100	TM50	TM100	P-value
SW (g)	2941.93	2872.80	2910.84	2858.95	2966.93	0.727
CCW (g)	1682.64	1626.28	1641.76	1643.29	1696.86	0.668
Dressing out (%)	57.20	56.58	56.41	57.56	57.13	0.529
RCW (g)	1404.24	1348.29	1368.10	1369.86	1358.85	0.937
Perineal fat (% RCW)	2.09	2.18	2.29	2.31	2.35	0.899

The obtained results showed that BSF fat and TM oil used as partial or total substitute of SBO can be used as feed ingredient in rabbit diets without impacting growth performance and slaughter traits. Future studies are needed to investigate the effects of dietary BSF fat and TM oil on meat quality traits and fatty acid profile.

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Response of piglets due to amino acid optimization of mixed diets with 75% replacement of soybean-meal by partly defatted insect meal *(Hermetia illucens)*

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Alternative protein sources, such as insect meals are in special focus of animal nutrition in order to replace soybean meal (SBM). As part of the multidisciplinary project, "sustainability transitions" this study investigated effects of replacing SBM by a partly defatted larvae meal (HM) from the black soldier fly (*Hermetia illucens*) in diets for piglets. HM is a protein concentrate (608.4 gCP/kg DM) and contains a well-balanced amino acid (AA) composition. The study aimed to evaluate the potential of HM as an alternative protein source for SBM diets for piglets with 75% replacement of SBM after weaning.

Methods: Immediately after weaning (21-day-old) 24 male castrated, modern genotype piglets [PIC 408 x (Large White x Landrace)] selected for equal body weights were divided into 3 experimental groups. Piglets were housed in individual flat deck cages for 5 weeks single feeding. Following an adaption period (14 days) with stepwise blending of the experimental diets with a commercial feed the experimental period lasted 21 days. Feed supply was semi ad libitum and water supply was ad libitum. The control diet (main ingredients: wheat, barley and SBM) contained 28% SMB. The two experimental diets replaced 75% of SBM by HM under study, both on a basic (crystalline L-Lys, DL-Met, L-Thr added) and an advanced level of AA fortification (crystalline L-Lys, DL-Met, L-Thr, L-Leu added) to achieve up to 105% of the AA supply of the control diet according to GFE (2006) recommendations (1). Response of piglets was weekly evaluated by zoo-technical parameters (growth, feed intake, feed conversion ratio, protein conversion ratio). Additionally, composition of gut microbiota was evaluated in the caecal content of piglets, sampled immediately after slaughtering. Digesta samples were collected in sterile bottles and cultured for several bacteria species (total bacteria count, Enterobacteria, *Clostridium* spp., *Enterococcus* spp., coliform bacteria, Lactobacilli, *Campylobacter* spp., *Salmonella* spp. and *E.coli*). One-way ANOVA (SPSS software package Statistics 24) connected with Tukey-test and Games-Howell-test identified significant differences between treatments ($p \leq 0.05$).

Results: Summarized results (**Table**) indicate that diet HM 75% +AA yielded similar results for all zoo- technical parameters under study as compared to the control diet. Diet HM 75% with the basic level of AA fortification tended to slightly lower growth, feed intake, feed and protein conversion ratio, respectively. However, no significant effect was observed. Accordingly, the composition of gut microflora was also not significantly influenced by the dietary treatments.

Table: Summarized results (Growth experiment about 36-57d) of the study (Mean ±SD)

Diets	Control (n=8)	HM 75% (n=7)	HM 75% + AA (n=8)
Final body mass (kg)	18.3 ± 1.26	17.1 ± 1.28	18.0 ±1.26
Feed intake (g/d)	652 ± 24	633 ± 41	641 ± 28
Feed conversion ratio (g/g)	1.43 ± 0.09	1.52 ± 0.15	1.45 ± 0.12
Protein conversion ratio (g/g)	0.31 ± 0.02	0.33 ± 0.03	0.31 ± 0.03

Conclusion: Partly defatted meal of *Hermetia illucens* is a promising alternative protein source for replacing SBM in diets for piglets.

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Insects fed with former foodstuffs for feed production: What are the risks to animal and public health?

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The need for proteins for human food and animal feed has increased over the last decades and is expected to increase substantially in the future. That is why there is a growing focus on less traditional sources of protein, such as cultured insects. Insects are a sustainable protein source, in particular when using organic residues and waste streams to feed them. But are they safe to feed to farmed animals when using former foodstuff as feed for the insects? To answer that question a desk study was performed 1) to assess the risks of the use of insects that are reared on a substrate composed of former foodstuffs as raw material for feed for food producing animals to animal and public health and 2) to indicate measures to control these risks. The most promising insect species for large scale industrial productions are black soldier fly, house fly, lesser mealworm beetle and yellow meal worm beetle. They have short reproductive cycles and their larvae can be cultured on low value organic residues or waste streams to produce high quality protein. The health risks that are associated with the use of animal feed derived from insects are determined almost exclusively by pathogenic micro-organisms and prions, that are introduced in the insect production chain via the former foodstuffs they are fed on. Insects can serve as vectors of these microbiological agents and can transmit them to the feed and food chains. The risks are determined by the origin of the former foodstuffs and the animal species of destination of the insects. When appropriate measures to prevent microbiological contamination of insects are taken, the feeding of food producing animals with feed derived from insects that are reared on a substrate composed of former foodstuffs poses no health risks for these animals and for humans consuming products from these animals.

Legal aspects concerning the use of edible insects in animal feed: Opportunities for a new business

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Problem Statement: According to the annual report published by the International Feed Industry Federation (IFIF 2016), by 2050 the demand for food will grow by 60% and between 2010 and 2050 the production of animal proteins is expected to grow by around 1.7% per year, with meat production projected to rise by nearly 70%, aquaculture by 90% and dairy by 55%. This poses big challenges for the feed sector, required to produce sustainable, safe and nutritious feeds for tackling the growing demand of animal protein in modern diets.

Edible insects can supplement traditional feed sources such as soy, maize, grains and fishmeal, providing a sustainable alternative to conventional feed proteins (FAO 2013). The Food and Agriculture Organization (FAO 2013) has pointed out the main advantages related to the use of insects as a source of protein in animal feed: high feed conversion rates, lower greenhouse gas emissions and the use for their production of less resources than more traditional sources of proteins in animal feed. Moreover, insects can be feed on bio-waste, compost and animal slurry, since they can transform waste into high-quality protein that can be use used for animal feed.

Despite the many advantages of using insects as proteins source, the use of insect processed animal proteins (PAPs) is currently allowed only for pets and fur animals. Article 7 of Regulation 999/2001/EC prohibits the use of PAPs as feed row material intended to be used for ruminant and non-ruminant animals other than pets and fur animals due the potential presence of prions which cause the transmissible spongiform encephalopathies.

This restriction has been amended by Regulation 56/2013 providing that aquaculture animals can be fed with animal proteins derived from non-ruminant and with compound feed containing such feed materials. Although, following this amendment, the use of insects should be allowed, the impossibility to fulfill the slaughtering requirements, put insects outside the scope of this provision.

The risk profile related to the production and consumption of insects as food and feed, has been assessed by the European Food Safety Authority (EFSA 2015). As regards the risks related to the presence of prions (connected to BSE), EFSA concludes that, compared to the occurrence of hazards in currently authorized protein sources of animal origin, the occurrence of hazards in non-processed insects is expected to be equal or lower, as long as the insects are fed on substrates that do not harbor material of ruminant or human (manure) origin.

Following this opinion, on November 2016 the European Commission has presented a draft regulation aimed at amending Annexes I and IV to Regulation (EC) No 999/2001 and Annexes X and XV to Regulation 142/201, allowing in this way the use of insects in feed for aquaculture animals.

Objectives: The primary goal of the paper is to clarify the European legislation currently applicable to the use of insects in animal feed, analyzing in detail the draft regulation and the novelties introduced by it. In particular, the legal consequences of the qualification of insects under the category of "farmed animals" will be addressed, pointing out the limits currently existing as concern the type of substrate used to feed them.

The paper will also analyzed critically the role of the European Food Safety Authority, its key role in assessing biological, chemical and environmental risks associated with production and consumption of insects and the impact of its opinion on legislative process.

Procedures/methodologies/approaches: The methodology chosen consists of a comprehensive literature review of scientific articles published in the last decade about the use of insects in food and feed, the analysis of European and national legislation related to the topic as well as the statements of national and European authorities appointed of issuing scientific opinions on the safety of insects.

Moreover, the paper will analyze the novelties introduced by the draft regulation amending Regulations 999/2001/EC and Regulation 142/2011/EC, which is expected to be published in the European Official Journal in July 2017.

Results: Preliminary results show how the use of PAPs in animal feed is strongly restricted at European level because the risk of BSE although some changes have been introduced for aquaculture animals. The results also show as strong barriers still exist to the use organic waste (catering and household waste) to feed insects, making their use as animal feed less competitive as concern the price. It follows that, in order to use them within a circular economy, amendments to rules currently in place are needed.

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Keywords: Insects, European legislation, feed legislation, Reg. 999/2001/EU, feed safety, circular economy

The relationship between the nutrient quality of black soldier flies and their rearing substrates

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Black soldier flies (*Hermetia illucens*) (BSF) are natural decomposers and have been used for waste management within the context of bioconversion. The larvae of BSF can reduce a significant amount of food, animal and sewage waste. BSF can be used as a protein source due to their ability to convert feed into edible products. The aim of this study is to measure the health safety and nutritional value of feed produced from BSF larvae grown on organic substrates. BSF larvae were fed on four different types of organic waste: poultry manure, brewery waste, cow dung, kitchen waste. Proximate analysis (content of: dry matter, ash, organic matter, fat, crude protein and neutral detergent fiber) study was carried out to determine the BSF nutritional value. Samples of the larvae and the substrates were analyzed 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 mycotoxins & aflatoxins. The data was subjected to analysis of variance (ANOVA) using statistical analysis system (SAS, version 9.1). Significance of means difference was done using general linear model (GLM) procedure. Bon-Tukey's was applied to separate the means at $p<0.05$ level of confidence. The results indicated variance in the nutrient composition due to the difference in the substrates. CP, EE, ADF and NDF were all affected by using different substrates. BSF are intended for use as protein sources in poultry and fish diets. They would be cheap replacements to the expensive protein sources such as fishmeal and oil seedcakes (e.g. sunflower meals/cakes) when reared on a suitable nutritive substrate.

Keywords: mycotoxins, protein value, bioavailability, feed

The importance of reliable input on consistent output in large scale production facilities

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Millibeter is an insect company founded in 2012 breeding the Black Soldier Fly (*Hermetia illucens*) to bioconvert organic side streams into chitin, lipids and proteins. We are currently upscaling our R&D facility to a full-scale commercial production unit. In this process we have been able to refine well-known breeding practices as well as develop new techniques.

One major achievement in working up to an industrial scale, has been the determination of an optimal density of larvae per bin and their optimal feeding regimes for certain feedstocks. These environmental conditions, along with temperature and humidity, guarantee a high and consistent output of larval biomass per bin. In any industrial setting consistency and continuity are key. When handling thousands of bins, they cannot be individually checked for their requirements and receive tailor made attention. Given a certain input, this results in a consistent and predictable output, which is essential for any industrial process.

Here we will explain the effects we saw of different larvae densities and feeding regimes on the processing capacity of the larvae in the bins, which can be extrapolated to the productivity of the plant in general.

It is important to note that every type of feedstock differs in nutritional value, consistency and dry matter content. In case of a new feedstock, it is hard to predict the larvae's processing capability, which implies that each feedstock has its own optimal feeding regime that needs to be determined, before it is introduced into a plant.

Lactic acid bacteria as a measure of bacterial diseases in insects reared for feed and food

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The integration of good management practice in insect production systems is important for a successful large-scale insect production. Management of diseases in insect production systems is especially important since the presence of insect pathogens generates a negative impact on the insect production. Lactic acid bacteria (*Lactobacillus kunkeei, Lactobacillus apinorum*; LAB mixtures) isolated from honey stomach of honey bees produce antimicrobial metabolites and peptides that can inhibit the activity of different insect pathogens. Therefore, such beneficial bacteria (LAB) can be considered for use as antibiotics in the management of diseases in insect production systems. We have conducted a pilot experiment to evaluate the antimicrobial activity of LAB on three insect pathogenic bacteria; *Serratia marcescens, Serratia plymuthica* and *Pseudomonas aeruginosa* and we obtained interesting preliminary results. Thirteen honey bee LAB strains were tested individually and in LAB mixtures.

Dual culture overlay assay was used to evaluate the antimicrobial activity of LAB against the tested insect pathogenic bacteria. The LAB strains were cultured individually in supplemented Man, Rogosa and Sharpe medium (sMRS) with 2% fructose and 0.1% L-cysteine and incubated at 35°C for 24 h. The cultures were centrifuged and the pellets were used to prepare suspension of each bacterial species. The concentrations were adjusted to 0.03 Optical density (OD) at 600 nm. LAB mixtures were prepared mixing equal volumes of the bacterial suspensions. Petri dishes with sMRS medium were prepared and a blank disk was placed in the middle of each petri dish. LAB suspensions were added onto the filter disc and incubated anaerobically at 35°C for 24 h. The insect pathogenic bacteria were cultured in LB or TSA and incubated at 28 or 37°C for 24 h. Cultures were harvested and suspensions of 10^8 cells/ml were prepared by mixing with their respective media and soft agar 0.8% at 42°C. This mix was carefully poured into petri dishes containing the discs with LAB and the petri dishes were thereafter placed in the incubator at 35°C for 24 h. The inhibition zone was measured in millimeters from the middle of the disc to the edge of the inhibition zone.

Some beneficial bacteria showed clear inhibition zones in the three tested insect pathogenic bacteria while others did not. In particular, *L. kunkeei* (Fhon2), *L. apinorum* (Fhon13) and the LAB mixtures had high antimicrobial activity. By showing that some of the LAB strain did inhibit insect pathogenic bacteria in vitro we suggest that they might be beneficial in large scale insect production. Next step will be to evaluate the LAB

strains effects on the insect host, *T. molitor* larvae, in combination with insect pathogenic bacteria.

Keywords: bacterial disease management, insects for feed and food, insect pathogenic bacteria, honey bee lactic acid bacteria.

Biorefinery of insects to high-added value products: Evaluation of fractionation procedures

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Recently there has been increased interest in the use of insects for food and feed. This is not only because of the high protein content in insect biomass, as also the lipid fraction has value. Chitin, a third major compound in insects (present in the exoskeletons), can be beneficial for feed/food in very low concentrations but affects the digestibility and the utilization of its macro and micro-nutrients in the present concentration. The chitin removal of bee extract for example increased digestibility of the proteins from 71 to 94%. Hence there is a need for separation technologies. The extracted chitin, protein and fat fractions can further be processed for various applications besides food or feed.

This poster presents a biorefinery for fractionating insect biomass focusing on preserving the functional properties of proteins. In this way, proteins do not only have nutritional value but can serve as functional ingredient. More specifically, an approach has been elaborated to fractionate larvae of the black soldier fly into chitin, lipids and protein fractions envisioning maximal valorization of the biomass for application in feed and chemistry. These larvae are well-known waste degrading insects, able to grow on different agro-industrial side streams and are on the list of the most promising insects for industrial production in the western world. Through the fractionation procedures high-value products like proteins, chitin and lipids can be recovered and thus turning waste into a second-generation resource.

Separating fat from animal residue is traditionally performed by rendering, a high temperature process resulting in denatured proteins. Our new fractionation procedures will be compared to the rendering process focusing on yield, purity and especially on preserving the functional properties of proteins. Furthermore, we will discuss different pretreatment and separation techniques to remove chitin. Results show that pretreatment as well as the solvent type has a major impact on the fractionation efficiency. This project has partially received funding from BBI Joint Undertaking under the European Union’s Horizon 2020 research and innovation program under grant agreement No 720715.

inVALUABLE: Insect value chain in a circular bioeconomy

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inVALUABLE is, to-date, the largest R&D project in Europe on insects as feed and food. The project involves 11 partners and runs from January 2017 to December 2019 with a total budget of 3.7M EUR. The vision of inVALUABLE is to create a sustainable resource-efficient industry for animal protein production based on insects. The partners span the entire value chain and include entrepreneurs, experts in biology (entomology and nutrition), biotech, automation, processing and food tech and -safety, as well as an international leading insect producer. This interaction of competences is key to lifting insect production to an industrial level.

Overall, inVALUABLE addresses three major challenges for the insect industry; 1) upscaling of production to industrial level, 2) regulatory issues and 3) consumer acceptance. Together with other large European R&D initiatives, trade associations and networks, inVALUABLE is expected to have a large impact on shaping the growing insect industry.

The presentation of inVALUABLE will give insight into the ongoing and future activities of inVALUABLE – reaching out to external stakeholders to build future collaborations. Initially, an overview of the project will be presented, which will be supplemented with some of the first results that have been generated so far. Below is provided some more background information on the goal and structure of inVALUABLE.

The goal is that inVALUABLE will facilitate Danish industrial insect production and be an enabler of new market opportunities for insects as feed, food and other high-value components. inVALUABLE aims to demonstrate the potential of using insects to meet the increasing demand for protein in the food chain by assessing the following specific objectives: 1) developing an insect value chain using low-value by-products - reintroducing valuable resources back into the food chain; 2) document the nutritional potential of insects using state-of-the-art animal models; 3) combine the best technologies to enable market penetration, focusing on large-scale production, automation and processing; and 4) support Danish/EU authorities on feed/food legislation providing data to ensure safe insect products.

inVALUABLE operates at an applied research level and is organized in three focal areas – Production (PRD), Processing (PRC) and Product Application (PAP) – comprising 9 work packages (WP); including project management which is coordinated by the Danish Technological Institute with a strong focus on dissemination of project activities. Production (PRD: WP1-3) will focus on optimization of the production of mealworms (WP1); improving the understanding of mealworm health and nutrition (WP2); and development of innovative technologies for implementing cost-effective production systems through automation and monitoring of mealworm health (WP3). Processing (PRC:

WP4-5) will develop processing methods of feeding substrates and insect biomass using different established and new technologies (WP4); and assess the feed/food safety of the obtained mealworm products as well as regulatory advocacy with relevant stakeholders (WP5). Product Application (PAP: WP6-8) focuses on how mealworm can be applied in feed and food products. WP6 will assess and document the nutritional and health value of such products by the use of state-of-the-art animal models; including the recommended assessment method 'DIAAS' for protein digestibility. In WP7, food application of mealworms will be considered including focus on sensory of insect-based products; while WP8 aims at influencing the market and public perception of insects as food through a diverse dissemination strategy including focus on consumer acceptance.

For more information see inVALUABLE.dk

Immunity of farmed insects: Basic and applied aspects

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The farming of edible insects has emerged as a promising alternative strategy for the production of protein-rich food and feed. The industrial production of insect-derived protein is more cost-effective and energy-efficient than livestock farming or aquaculture, and results in a lower ecological footprint. However, mass rearing of farmed insects and microbial contamination of their diets promote the spread pathogens and parasites. The use of antibiotics in livestock farming has been recognized as a major reason for the increasing prevalence of antibiotic-resistant human pathogens. The presentation aims to expand the advantages of insect farming versus livestock farming beyond economic arguments and a beneficial ecological footprint to include options enabling industrial protein production without the reliance on antibiotics.

Insects possess a potent innate immune system which encompasses the synthesis of a broad spectrum of antimicrobial peptides. These immunity-related effector molecules contribute to the control of the insect gut microbiota, the sanitation of the gut prior to pupation and to self-medication. The presentation will briefly summarize our current knowledge about innate immunity in farmed insects or closely related model insects, and focus on features that are promising for the development of strategies to prevent the outbreak of infections without the application of antibiotics thereby achieving beneficial carry-over effects for the consumer. Recent insights into trans-generational immune-priming and nutritional immunology of farmed insects such as the Black soldier fly *Hermetia illucens* and the mealworm *Tenebrio molitor* will be highlighted. These findings are important to understand the role of the insect gut microbiota and its manipulation by addition of beneficial microbes to the diet. The potential use of probiotics to strengthen the pathogen resistance of farmed insects will be discussed.

COST Action: A comprehensive tool for networkers

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COST Actions are a flexible, fast, effective and efficient networking instrument for researchers and stakeholders to cooperate and coordinate nationally funded research activities. They are bottom-up, allowing researchers to jointly develop their own ideas in any science and technology field. The funding available can be used in a range of networking tools, such as workshops, conferences, training schools, short-term scientific missions (STSMs), and dissemination activities. COST Actions are selected via a competitive Open Call and proposals are evaluation is fully science and technology-driven, ensuring a simple, transparent and process. COST has an Excellence and Inclusiveness Policy looking for actively engage young researchers and those from less research-intensive countries.

This talk will provide a short-overview on COST and how to submit proposals for COST Actions.

High-value compounds delivered from insects grown on waste

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In addition to increasing population, limited land and water, and increasing costs of fertilisers and energy, one of the biggest challenges of food production is wastage. Organic waste management is a complex issue involving social, economic and environmental aspects. About 30% of all produced food is wasted in pre- or post-consumption. Annually 250 billion litres of water and 300 million barrels of oil are used to produce 1.3 billion tons of food worth 750 million $ that is never eaten (FAO, 2013). High-value products can be produced from these materials using insects. Insect larvae, in particular, or their components, have the potential to become prime ingredients in food and animal feed.

Essential fatty acids, minerals, vitamins and amino acids occurring in significant amounts in the larvae body, are susceptible to modification based on their feeding material, allowing process tailoring for specific products. In addition, co-extraction of high-value compounds, such as antimicrobial and antifungal agents or chitin, have the potential to significantly increase the cost efficiency of the overall process.

The aims of this study was to develop a close-loop system using mild conditions were *Hermetia illucens* larvae grown on organic waste, were extracted using a sustainable cascade process allowing the contemporary separation of proteins, lipids and chitin.

Hermetia illucens was chosen as most suitable candidate due to its non-pest status and it predisposition to process variety type of waste with high converting efficiency (up to 60%).

Preliminary results showed that larvae composition varies with age, mostly for the protein-lipids ratio which varied from 3.3 to 2.3 and that different lipids can be fractionated at different stages of growth.

In addition, the use of different extraction techniques at different growth stages, suggests that the process can be tailor to produce the best larvae type-product ration.

Conditions and path of close-loop extraction system was designed and optimised. In addition to high lipid and protein content significant amounts of carbohydrates were detected at different stages of growth. This suggests the further evaluation of carbohydrates usage, for example for bioethanol production, would be worth considering.

The Mediterranean fruit fly, *Ceratitis capitata* – agricultural pest and mass-producible protein source

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The Mediterranean fruit fly, *Ceratitis capitata*, is a major destructive larval insect pest throughout the world due to its broad host plant range that includes more than 260 different fruits, flowers, vegetables, and nuts. It exhibits a unique ability to invade and adapt to ecological niches throughout tropical and subtropical regions, exhibiting a wide range of host preferences. It is one of the primary insect pest species to be controlled by the sterile insect technique (SIT), and has become one of the most intensively studied non-drosophilid insects in terms of genetic analysis and manipulation due, in large part, to efforts to create strains for improved SIT efficacy. The SIT is an environmentally friendly method that can suppress a pest population by the release of sterilized male individuals, which lead to sterile matings and no subsequent progeny.

The SIT in general is dependent on effective `sexing techniques', that are used to separate females from males, so that only males can be sterilized and released. Novel technologies on the sterilization method and the marking of flies that are released are important to improve the fitness and monitoring procedure after a field release. Novel technologies on all three aspects will be presented. While these techniques are important for medfly pest control and a transfer of the SIT to other species, the mass-production that is needed and established for producing the insects for SIT programs, could be used in other fields. The options of using the insects for food and feed production are discussed.

Use of Insect fats in cosmetics

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Research on insects is mainly focused on food and feed, but in addition, insects may provide an environmental-friendly way of producing high quality bio-based materials that can be implemented for non-food/feed applications. Insects can be bred on organic waste, in high numbers, and on small surfaces, therefore making large scale industrial breeding possible. Moreover, some insect species are capable of efficiently converting organic waste into valuable biomaterials. In the RADIUS lab of the Thomas More University College, research aims to identify and demonstrate the use of several insect species in industrial applications. Insects are reared and the chitin, protein and fat fractions are subsequently isolated. For each of these fractions, non-food/feed applications are identified.

Here, we describe the potential use of fats extracted from black soldier fly (*Hermetia illucens*), the house cricket (*Acheta domesticus*) and the locust (*Locusta migratoria*) in cosmetic applications, more specifically the use in hand creams. The extracted fats were qualitatively characterized by TLC and the free fatty acid content was determined. The fatty acid profiles were determined by GC-MS. The fats of all 3 species were used in hand cream formulations which were evaluated for stability, viscosity, polarity, spreadability and potential interference with preservative systems. We conclude that fats from the cricket and the locust but not from the black soldier fly are suited to be used in a hand cream formulation. However, better refinement of the fats is needed to make them more suited for cosmetic implementation.

Keywords: Black soldier fly, house cricket, locust, cosmetics, hand cream, fats

Bioprocess engineering aspects of insect cell expression systems

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Insect-based expression platforms are widely used for the laboratory-scale production of recombinant proteins, but are also suitable for industrial-scale processes. Today, the most popular insect cell lines for protein expression are derived from *Spodoptera frugiperda* (Sf9 and Sf21), *Trichoplusia ni* (High FiveTM), *Bombyx mori* and *Drosophila melanogaster* (S2). Recombinant protein production in S2 cells is achieved by stable transformation [1,2]. The other cell lines are used to host the baculovirus expression vector system (BEVS), mainly Nucleopolyhedrovirus (NPV) like *Autographa californica* (AcMNPV) or *Bombyx mori* (BmNPV). Thereby, AcMNPV is the more versatile tool, as BmNPV has a narrower host range and is limited to *B. mori* cells [3]. For the production of recombinant proteins, the two most popular systems are the S2 cells and the baculovirus expression vector system (BEVS) using AcMNPV, which remains the gold standard in insect-based expression systems for recombinant proteins [1,4].

The industrial application of protein expression systems is linked with efforts of maximizing the yield of high quality protein. In respect of their individual nature, both mentioned insect expression systems pose challenges for optimization. Some of these challenges will be discussed based on the following two model cases of bioprocess engineering approaches.

Although more than 30 years of development to maximize the stability and reproducibility of the production of high-quality therapeutic proteins, the BEVS still is dealing with drawbacks such as the lytic infection cycle and the shear sensitivity of infected insect cells. The effect of air bubbles and turbulence on shear sensitivity has been described theoretically but is not completely understood in practice. Smaller bubbles were formerly assumed to be more harmful than larger ones, but we found that cell damage is also dependent on the concentration of protective agents such as Pluronic® in the culture medium. At the appropriate concentration, Pluronic® forms a layer around air bubbles and hinders the attachment of cells, thus limiting the resulting damage. In this context, we used micro-aeration to generate different bubble sizes and confirmed that size is not the most important factor, but rather the total gas surface area in the reactor [5]. If the total gas surface area exceeds a certain threshold, the concentration of Pluronic® is no longer sufficient for cell protection [6,7]. To investigate the significance of shear forces, a second study was carried out in which infected insect cells were cultivated in a hollow

fiber module to protect them from shear forces. Both model studies revealed important aspects of the design and scale-up of BEVS processes for the production of recombinant proteins.

Transfection of S2 cells with the desired genetic construct results in a population of heterogeneously expressing the protein of interest due to the variation of insert loci and frequency. Based on the production of an antimicrobial peptide (Gloverin-family), derived from the greater wax moth *Galleria mellonella*, we describe the efficient heterologous expression and basic characterization of the peptide [8]. Thereby, a holistic strategy was pursued comprising optimizations on cellular and process level. Employing single cell dilution resulted in highly productive clones and an increase in yield by 100% compared to the original polyclonal *Drosophila melanogaster* S2 cell line. Additionally, comprehensive screening for suitable expression conditions using statistical experimental designs revealed that optimal induction was achieved using 600 µM CuSO4 at the mid-exponential growth phase. Under these conditions, 25 mg/L of the AMP was expressed in a 1-L bioreactor, whereby optimal induction and harvest times were ensured by dielectric spectroscopy and online measurement of optical density. The resulting gloverin was purified and showed specific antimicrobial activity against two different strains of *Escherichia coli*.

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Keywords: baculovirus expression vector system, shear stress, stably transformed *D. melanogaster* S2 cells, recombinant protein expression, online process monitoring

Valuing mulberry silkworm: an opportunity to revive French silk production

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Edible insects have always been part of the human diet. But new challenges revolving around satisfying growing global needs for food and feed, especially proteins, places them at the heart of strategic thinking on alternative sustainable farming practices. It is estimated that more than 2000 insect species are consumed globally, most of them being collected in natural forests.

Even though most of the edible insects are gathered, often in forests, insect farming is getting more and more popular. Innovations in production methods for this source of proteins have appeared in many countries, ranging from developing better collection techniques to mass rearing of certain species, including small, rural, family owned, semi-farming units.

Feeding this new type of livestock is a major challenge. Currently, the solution being favored in Europe consists in using agricultural products and byproducts. This solution poses numerous technical issues, including two main ones: their safety for farming (presence of pesticides) and their cost of procurement/production. Considering species diversity and associated feeding mechanisms, other less expensive feed resources can be utilized. Aerial biomass from the trees is one of them, abundant and traditionally used notably to raise Lepidoptera larvae who are usually phytophagous.

It is the case in silkworm farming for sericulture with white mulberry leaves (*Morus alba* L.). The silk industry, which used to be flourishing in France, almost disappeared from the Hexagon circa 1860. Today, Chinese and Indian productions cumulatively total 50% of the World silk production, while French production is anecdotal. Many factors explain that decline, one of them being the loss of profitability of the industry caused by production costs having increased too much and lack of competitiveness.

Similarly to what is done in other producing countries such as China or Thailand, utilizing another byproduct important to the industry, the silkworm pupa, could be an opportunity to restart it from scratch. Developing a national market, either for recreative and festive human food or more traditionally animal feed from a traditional activity created new business opportunities. Such a revival could have major impacts in terms of creating local rural jobs, modifying the landscape and revitalizing rural landscapes which are currently in crisis.

The article will focus on demonstrating possible perspectives, limits and constraints faced in developing such an industry (regulatory, cultural, technical) at a local scale (Cevennes – Occitanie)

Keywords: Edible insects, insect farming, innovative proteins, livestock byproducts, silk production, silkworm, circular economy, *Morus alba* L., *Bombyx mori* L.

Impact of blanching, industrial microwave drying and freeze drying on the nutritional quality, the microbial quality and the browning index of mealworm larvae (*Tenebrio molitor*)

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Introduction: Yellow mealworm larvae are being produced at industrial scale for food and feed purposes. To allow storage and transport in the logistic chain, the insects must be stabilized after harvest. Currently, the larvae are blanched as a killing step and then frozen or freeze dried. The aim of this research was an in depth investigation of the possibilities of industrial microwave drying as an alternative to freeze drying. The impact of blanching, microwave drying and freeze drying on the nutritional quality, the microbial quality and the colour of the larvae was evaluated in three experiments.

Materials and methods: To compare microwave and freeze drying, in a first experiment larvae from the same production batch were dried in an industrial band microwave dryer (MEAM Dry 32, Herk-de-Stad, Belgium) or in a freeze dryer (VirTis Sentry 5SL, Geel, Belgium) according to process parameters which were optimized in preliminary experiments. Moreover, each technology was applied both without and with a blanching step prior to the drying step, to evaluate the impact of blanching. After drying, the water activity (LabMaster aw, Novasina, Lachen, Switzerland), the moisture content (oven method: 17h at 105°C), fat content (Soxhlet), fat oxidation (peroxide and p-anisidine values), protein content (Kjeldahl), ash content (dry ashing) and vitamin B12 content (Vitafast® testkit) were measured. The colour of the samples was measured immediately after drying and during four months of storage (spectrophotometer CR-5, Konica Minolta). In a second experiment, the hypothesis stating that the application of vacuum during microwave drying results in a better end product quality than microwave drying alone was investigated for mealworms. This was done in a pilot microwave dryer (μWaveVac0150, Püschner, Germany). Larvae from the same batch were dried using the two approaches. For both techniques almost the same drying time was used, but in the test with vacuum a lower microwave power was applied than in the test with microwave drying alone. The quality of the dried products was compared based on the parameters mentioned above. In a third experiment, larvae were either blanched (during 40 sec), or microwave dried (during 10 min) in the industrial facility mentioned above (MEAM), or blanched (during 40 sec) and then microwave dried (during 8, 10, 13, 16, 20 min). After the treatments, the moisture content, the water activity and a series of microbial parameters (total aerobic count, aerobic bacterial endospores, Enterobacteriaceae, lactic acid bacteria, yeast and moulds) were assessed according to the ISO standards assembled by DIJK *et al.* (2015).

Results and discussion: When microwave and freeze drying were compared, similar moisture contents and water activities were obtained by both techniques. Protein, fat and ash contents were almost not affected by drying itself, nor by the drying method. Primary oxidation was low (i.e. below 5 meq/kg) for fresh, blanched and microwave dried samples, but it was significantly higher for freeze dried samples (125 and 55 meq/kg for freeze dried and blanched plus freeze dried samples, respectively). Secondary oxidation was low (anisidine values below 3.5 in all samples). Vitamin B12 content was not affected by blanching, freeze drying or a combination of both and ranged between 0.64 and 0.85 µg/100 g DM. However, microwave drying, either or not combined with blanching, resulted in a significantly lower vitamin B12 content (0.31 µg/100 g DM for both types of samples, blanched or not). In contrast, the browning index and the colour of microwave dried mealworms remained constant during the tested storage period of four months, whereas freeze dried samples showed more browning as storage time proceeded. For heat sensitive foods, the application of vacuum during microwave drying allows to use a lower power, which may lead to lower product temperatures and a better preservation of product quality during drying (DROUZAS & SCHUBERT 1996). For mealworm larvae, surprisingly, no differences in quality parameters were found, except for fat oxidation which was higher when vacuum was applied than when it was not used. Vitamin B12 and colour were exactly the same in the two types of dried products. The experiment that focused on blanching and microwave drying yielded the following findings. Blanching for 40 s followed by industrial microwave drying for 8, 10 or 13 min did not yield larvae with a water activity below 0.60, which is necessary to prevent microbial growth during storage of the product. Drying times of 16 or 20 min yielded average water activities of 0.16 and 0.23, respectively. The number of vegetative cells was reduced to a large extent by blanching plus drying, but the number of bacterial endospores hardly decreased. Total viable counts after treatment were maximally 3.4 ± 0.8 log cfu/g for all samples, whereas initial counts were above 7.8 ± 0.3 log cfu/g. Microwave drying without a prior blanching step reduced all microbial numbers to the same extend as blanching plus drying, except for Enterobacteriaceae. Apparently, in terms of microbial reduction, blanching does not provide added value on top of the inactivation by microwaves, which is likely due to a combination of a temperature increase in the matrix and electromagnetic effects (VANDEWEYER *et al*. 2017).

Conclusions: Microwave drying is a valuable alternative to freeze drying for drying mealworm larvae. In general, a similar end product quality is obtained, except for the vitamin B12 content which is better preserved during freeze drying. The advantages of microwave drying are that the treatment time is shorter and that, in contrast to freeze drying, no browning of the product occurs during storage afterwards. Although sustainability and cost of both technologies were not investigated in this study, it is likely that microwave drying is a more sustainable and cheaper process than freeze drying. The application of vacuum during microwave drying does not result in additional benefits, and hence, mealworm processors are advised not to invest in the vacuum option when considering the purchase of a microwave dryer. Regardless of treatment times, blanch-

ing alone or blanching plus microwave drying represent a pasteurization treatment, i.e. killing vegetative cells but not or hardly any spores.

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Enzymatic and non-enzymatic causes of browning during grinding of insects: *Tenebrio molitor*, *Alphitobius diaperinus* and *Hermetia illucens*

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Insects are more acceptable as alternative protein source for Western consumers when insects are not recognized as such. To make the insects not recognizable as such, grinding is necessary. During grinding of insects, undesired browning can occur. The goal of this research was to find the causes of this brown colour formation during grinding of the larvae of three insect species: *Tenebrio molitor, Alphitobius diaperinus* and *Hermetia illucens*. The focus was on enzymatic browning and the influence of minerals on the colour formation.

During enzymatic browning, polyphenols are oxidized by enzymes to quinones which can subsequently lead to brown colour formation. Different enzymes, i.e. tyrosinase, tyrosine hydroxylase, L-DOPA decarboxylase, laccase and peroxidase can be present in insects, and these all can play a role in enzymatic browning during grinding. Tyrosinase appeared to be the most important enzyme responsible for enzymatic browning during grinding of insects.

When comparing the three insect species, it was shown that the brown colour formation can also be caused by factors other than tyrosinase, as *H. illucens* showed the darkest colour, but the lowest tyrosinase activity.

Therefore, the influence of mineral composition on brown colour formation in *H. illucens* was investigated. Different metal ions were tested for their ability to form complexes with polyphenols. From the different metal ions, iron showed complex formation with polyphenols and gave a dark colour in line with the colour of *H. illucens*. The iron content in *H. illucens* was 221±6.07 mg/kg based on dry weight, which is significantly higher compared to the iron content of *T. molitor* (61±9.71 mg/kg) and *A. diaperinus* (54±1.72 mg/kg).

During grinding, minerals could form a complex with polyphenols. Different structures of polyphenol-iron complexes were characterized using mass spectrometry, e.g. a tris-DOPA-iron complex.

In conclusion, this research showed that tyrosinase is the enzyme responsible for enzymatic browning during grinding of *Tenebrio molitor* and *Alphitobius diaperinus*. Furthermore, the presence of iron is an important factor for the dark colour in *Hermetia illucens* during grinding.

Safety aspects of edible insects for use in the food and feed value chain - investigation of bioaccumulation of contaminants and process-induced mitigation of allergenicity

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Recently, edible insects have emerged in Western countries as snack product and novel source of animal-derived protein and caloric energy for livestock husbandry and human nutrition. The safety concerns associated with the introduction of insects and products thereof in the feed and food value chain and the resulting research needs were clearly pointed out by national European authorities and the EFSA. Beside the potential microbiological hazards, especially risks associated with the bioaccumulation of contaminants as well as the allergenic potential of insect proteins were addressed in those risk profiles.

This presentation will give an insight into bioaccumulation of environmental contaminants by Black soldier fly larvae (*Hermetia illucens*) and the process-induced reduction of the allergenicity of migratory locust (*Locusta migratoria*) proteins.

In the first study, newly hatched black soldier fly larvae were reared on substrate spiked with heavy metals (As, Cd, Cr, Hg, Ni, Pb), mycotoxins (aflatoxin B1/B2/G2, deoxynivalenol, ochratoxin A, zearalenone) and pesticides (chlorpyrifos, chlorpyrifos-methyl, pirimiphos-methyl) under constant breeding conditions (10 d, 28°C, 67% RH). The extent of contaminant bioaccumulation in the larvae as well as the effect on growing performance was examined. Heavy metal substrate contamination was found to impair larval growing indicated by lower post-trial larval mass and feed conversion ratio (FCR). For cadmium and lead accumulation factors of 9 and 2, respectively, were determined. In contrast, mycotoxins and pesticides were neither accumulated nor did they affect the growing performance in comparison to the control. In the second study, the effect of processing such as heat treatment (cooking, autoclaving), enzymatic hydrolysis using different commercial proteases or combination thereof, and fractionation procedures on IgE-binding of migratory locust protein was investigated using sera of crustacean- and house dust mites-allergic patients. Interestingly, enzymatic hydrolysis and heat treatments of locust protein extracts were able to reduce IgE-binding revealing successful mitigation of allergenicity by processing.

The results of our experiments evidence on the one hand that the use of edible insects as livestock feed requires adequate contaminant monitoring to ensure feed and food safety along the value chain. Especially cadmium and lead need to be controlled in the substrates as well as in feedstuff containing insects. On the other hand, it could be shown that certain food processing methods such as enzymatic degradation and heat treatments can be a promising approach to reduce the allergenic potential of edible insect proteins.

Practical & technical considerations for use of insects within food product development

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Insects have been touted as one of the primary food resources that could help drive a more sustainable and ethically conscious future. Publications from large governmental bodies such as the Food and Agricultural Organisation of the United Nations (FAO) have drawn major interest from businesses both large and small. This rush to be the first to market has driven a research vacuum, where the companies already in production around the world, are running blind on subjects like optimal production processes and the practical aspects of product development.

The aim of this research was to consider some of the technical attributes that adding insects into foods can bring and discuss how these attributes may affect consumer acceptance. Microscopy demonstrated that commercial cricket flour has particles above the size threshold for mouthfeel, reinforcing textural barriers to acceptance. The particle shape and the distribution of the chitin exoskeleton throughout commercial cricket flour has negative implications with bakery products and other foods which rely on the formation of gluten. Cricket flour has also been shown to be supportive of microbial life, potentially having impacts on shelf life and product safety. Moisture absorption may create textural issues over the shelf-life of a product. The colour created when adding cricket flour into a product matrix has also been found unpalatable but clever ingredient usage can go some way to combatting this issue.

It is expected that negative attributes in insect-based food products would create a higher negative response due to this reinforcing a pre-determined expectation for the negative. Understanding the technical attributes and how the insect flour behaves in a product should allow for the creation of better products.

High-low quality feeding regime to improve *Gryllus bimaculatus* (black cricket) survival on bio-waste

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Previous papers frequently claim that insects are the ideal solution to several food security hurdles, one of which being the processing of food waste. While it has been demonstrated that some insects survive well on bio-waste (e.g. *Hermetis illucens*), no study has to date demonstrated success with the more common species for human consumption, crickets. In other livestock sectors it has been established that nutritional requirements vary with age of animal and diet must be altered accordingly to achieve best growth, e.g. chick feed to layer mash in chickens. This trial aimed to establish if a similar feeding regime of high to low quality feed can improve the survival of crickets on bio-waste products, by establishing a better base metabolic rate. Pilot trials demonstrated poor to no survival on beer waste and cow manure and mid-level survival on unprocessed vegetable waste with chicken feed as the control feed stuff. Based on this feed regimes of either 1 or 2 week high quality feed (chicken feed) and then either 2 or 3 weeks of low quality feed (beer waste) or medium quality feed (vegetable waste) were tested. Results indicate that even 1 week of high quality feed makes a significant difference in survival and end size on low-quality bio-waste. More detailed results of exact impact of feed on growth and survival will be presented. However, this suggested that it may be possible to reduce the reliance of the commercial insect sector on chicken feed.

GREEiNSECT: Opportunities and challenges for insect farming for 'green economy' in Kenya

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The expectations to insects as the environmentally sustainable solution to feed world population have exploded in the recent years. To address the questions of opportunities and challenges in turning the potential of insects into solutions, University of Copenhagen was granted to coordinate 1,3 mill Euro in support for the project 'GREEiNSECT – insects for green economy'. The grant was awarded for 2014-2017 by Danida, Ministry of Foreign Affairs of Denmark, for collaborative research and research capacity building in Kenya. Within GREEiNSECT, research has been conducted on two main insect production systems: small- and medium scale cricket farming for food and small- and large scale Black Solider Flies (BSF) for animal feed. A total 7 PhD students are supported through GREEiNSECT in research topics related to insect production, feeding, insect product development, consumer perception, nutritional impact, environmental impact (LCA) and impact of livelihood. Cricket well as BSF production systems are both in an early phase of implementation in Kenya, and GREEiNSECT has contributed to place Kenya as a regional center for insect for food and feed. The presentation will include an overview of key research results, and on the importance of supporting research capacity building.

Insects & the food scenario analysis by the global council on food security and Agriculture at the World Economic Forum

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Protix produces insect based ingredients with the highest focus on quality and reliability. Healthy, nutritional and natural, insects will play a vital role in a food secure future without depleting our planet. Protix enables food in balance with nature for all living creatures.

www.protix.eu

Industrializing the production of Black Soldier Fly larvae for animal feed

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Bühler Insect Technology Solutions (BITS) provides modular solutions for the industrial-scale production of quality insect ingredients, covering the entire supply chain from feedstock handling to rearing, separation and extraction. BITS is a joint venture between Protix, the leading insect company, and Bühler, the technology group for food, feed and advanced material processing.

Link: www.buhlergroup.com/insects

Effectiveness of different information on consumers' willingness to taste insect products

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Understanding external influences on consumer behavior is fundamental for the successful marketing and promotion of insect products in Europe.

Insect products bear a great potential for the transition towards more sustainable food systems of western societies (RUMPOLD *et al.* 2017). Edible insects are a source of protein, fats, vitamins, minerals and energy (RUMPOLD & SCHLÜTER 2013, VAN HUIS 2013) and emit fewer GHG emissions than other livestock and can be fed from organic waste (VAN HUIS 2013).

Consumer eating behavior is complex and influenced by multiple determinants assignable to personal factors such as values or habits but also situational influences such as the framing of advertisement messages. The objective of the study is to investigate the effectiveness of different pieces of information regarding different aspects of entomophagy.

A tasting of insects coupled with an inquiry of participants of a public science night in Berlin, Germany (Lange Nacht der Wissenschaften Berlin 2017) has been performed. In addition to socio-demographic information (age, gender, cultural background, educational level) of the participants, their eating habits (vegetarian, vegan, omnivorous), their perception of own open-mindedness towards new things in general and their previous experience with entomophagy was enquired. In particular, their willingness to eat and buy insects in different forms (dried whole, ground as an ingredient in a bar) was enquired and tested. Up to here, no frame was used to influence respondents' behavior.

After the inquiry, the participants were offered freeze-dried whole mealworms (*Tenebrio molitor*) and locusts (*Locusta migratoria*). If the inquiry did not end with a sampling of the offered insect products by the participant, a single piece of positive, negative or neutral information was provided e.g. concerning nutrition, risks, taste, and tradition of edible insects.

It was recorded whether the respective information resulted in a sampling of the insect products. Based on these findings, it was determined if specific additional information could evoke a change in consumer acceptance. Furthermore it was evaluated which information had the highest impact.

As a side note, it has to be considered that due to the setting of the inquiry during a science night, the target audience might not be representative since most visitors of such

events have an above-average educational background and are in general more open-minded and inquisitive.

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Poster Session

Study on artificial diets for the rice grasshopper, *Oxya chinensis sinuosa*

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This study is the report on the rearing of the *Oxya chinensis sinuosa* on an artificial diet. We fed rice grasshoppers kind of four protein or kind of three carbohydrate. Mortality rate was low at soybean meal, fish meal, whole grain wheat powder(WGWP) and whole grain rice powder(WGRP). The artificial diets consisted of whole grain powder from one of two Gramineae plants, soybean meal or fish meal, dried yeast, milk casein, vitamin mixture, agar etc. and water.

A feeding is soybean meal or fish meal of 2% or 5% with one of whole grain powder. Adult weight is heavier at 5% than 2%. Mortality rate was lower at soybean meal than fish meal and lower at 5% of WGWP or WGRP than 10% to 15%. It's possible to rear rice grasshoppers with WGWP or WGRP instead of dry leaves powder.

Keywords: *Oxya chinensis sinuosa*, rice grasshopper, artificial diet, mortality rate

Microbiological and chemical changes during storage of edible insects

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Introduction: Since insects have been introduced as an alternative food product in human diets in Europe, regulations on microbiological and chemical criteria are necessary. Therefore investigations to evaluate the influence of manufacturing, handling and distribution processes on microbial and chemical quality of edible insects have to be carried out. The aim of the present study was to gain insight into microbiological and chemical changes during storage and packaging of edible insects.

Animals, material and methods: Mealworms (*Tenebrio molitor*) and locusts (*Schistocerca gregaria*) from Austrian insect production were analysed in term of quality changes. Insects were heated in water bath at 80°C for 5 minutes. The ratio of insects to water was one to ten. After cooling the insects were packed anaerobically under modified atmosphere (30% CO2, 70% N2) or aerobically wrapped in oxygen permeable foil. The samples were stored at 4°C for three weeks. Microbiological analyses were performed by standard plate count methods. Total aerobic mesophilic counts, Enterobacteriaceae, lactobacilli, pseudomonades, staphylococci, yeasts and moulds were enumerated. Fat oxidation has been evaluated by measuring thiobarbituric acid reactive substances (TBARS). TBARS were determined according to WITTE *et al.* (1970). Biogenic amines the biogenic amines were separated as dansyl derivatives by RP-HPLC (PAULSEN *et al.* 1997).

Results and discussion: Initial aerobic mesophilic counts (AMC) on mealworms and locusts ranged from 5.3-7.3 log10 KBE/g. Heat treatment resulted in a 7 log reduction of AMC in mealworms and 5 log reduction in locusts. The total bacteria count remained steady under anaerobic storage, while it increased under aerobic storage up to 6 log colony forming units. According to FERIOLI *et al.* (2008) in the case of meat sensory deterioration of quality has been determined when TBARS value is between 1 and 2 mg malondialdehyde/kg. Mealworms showed an initial value of 0.6 (average of 5 determinations) and around 1 during 3 weeks of storage. The results show no or only marginal differences between aerobic or anaerobic storage. The initial values of locusts were about 4 times higher than values from mealworms. During aerobic storage there was no change within the first two weeks followed by an elevation nearly twice the number. The reason for the differences in the initial values cannot be explained by neither the amount of fat nor the fatty acid composition. The fat concentration is more or less equal and lies about 38% in dry matter, the percentage of unsaturated fatty acids is slightly higher in mealworms than in locusts. A possible reason could be the intake of oxidized fat. Bio-

genic amines are either decarboxylation products of amino acids and are formed during spoilage or fermentation. On the other hand some of them play a role in metabolism and are responsible for cell growth and apoptosis and called polyamines. In mealworms and locusts under investigation only putrescine, spermine and spermidine have been found. Other biogenic amines like histamine, thyramine or cadaverine could only be determined in small amounts of not higher than 50 mg/kg in some samples. In contrast to this, putrescine, spermine and spermidine are found in all samples and higher amounts. In mealworms and locusts each spermine and spermidine are present in concentrations of 50-100 mg/kg. As expected, the concentration did not increase during storage because - as in other foodstuffs - these two polyamines are not formed during storage or fermentation. Putrescine is considered as biogenic amine and as polyamine, because putrescine is formed during spoilage and has physiological functions. In mealworms 100 to 400 mg putrescine has been found without increase during storage. In locusts the values laid between 1000 and 1500 mg/kg and no change could be observed during storage. The difference between mealworms and locusts can be explained by the bigger volume of the intestine in locusts, because putrescine can also be formed by bacteria in the intestine. The reason of the variation between the individual samples could be different amounts of amines in the individual insects.

Conclusion: Under anaerobic conditions mealworm and locust can be stored for three weeks at 5°C without microbiological or oxidative deterioration of quality. Nutritional and sensory analyses have to be included in further investigations to ensure the maintenance of product quality.

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Processing of cricket flour for protein extraction

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Alternative proteins are of interest as a protein crisis is envisaged due to the rising pressure on the food supply. Many insect species have proven to be a great source of high quality sustainable protein, albeit unexploited by the developed world for cultural reasons: therefore novel food formulations are needed to meet the preferences of western consumers. The present study focuses on a commercial flour obtained from a suitable species, the Banded Cricket *Gryllodes sigillatus*. Defatting was performed with both soxhlet (hexane) and decanting (ethanol), and compared to the label. The crude protein content was 58.3 ± 0.2%, which increased to 79.1 ± 0.3% after ethanol defatting. These two batches of cricket flour and ethanol defatted cricket flour were further processed through isoelectric solubilisation/precipitation in order to isolate the soluble protein fraction. Two pH ranges were subsequently used, one to solubilise the protein and the other to precipitate it. pHs 5 - 12 were tested for the solubilisation, showing a maximum at pHs 10 - 12. For the precipitation the pH range 2 - 7 was investigated, showing higher yield around pHs 2 - 3. The recovery of a functional and soluble protein fraction provides a possible alternative protein for the development of novel food products.

Observation of the effect of high-density rearing and yeast as feed stimulus on growth performances of *T. molitor* larvae (Coleoptera, Tenebrionidae) under mass-rearing conditions

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Optimisation and automatization of mass rearing for the pest insect *Tenebrio molitor* (Coleoptera, Tenebrionidae) are in high demand to maximize the production of high-quality insect protein. Under this pretext, we analysed the effect of growth and biomass accumulation of *T. molitor* larvae with different yeast concentrations in the diet. Few reports exist about the effect of yeast as feed stimulus (FRAENKEL & BLEWETT 1943, LECLERCQ 1950). Thus we measured the food conversion (EDC), ingested food conversion (ECI) and gained weight of larvae biomass per food consumed with different yeast concentrations over three consecutive 3-week periods to analyse the relationship between growth performances and different yeast concentrations in the diet.

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Alternative possibilities of noninvasive monitoring systems for the industrial rearing of *Tenebrio Molitor* larvae

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The Food and Agriculture Organisation of the United Nations and the World Economic Forum have pointed out the risks related to increasing demand for freshwater, energy and food in the years to come (FAO 2014, WEF 2014). Due to a rising world population and the increasing demand for meat, the need for protein resources in livestock breeding becomes a challenge of advancing relevance. Common protein sources like soybeans or fish meal are no longer sufficient for a sustainable supply causing environmental and economic damages (FEARNSIDE 2001). An alternative protein source are edible insects, which are increasingly attracting worldwide attention for livestock breeding and also prospectively for the human consumption. In this regard, a major task is the development of automation processes for the industrial rearing to produce large quantities of high quality insects continuously. Currently, there are already a few industrial scale enterprises, for example AgriProtein with 1 ton larvae per day, but the level of automation is still quite low or virtually non-existing. Most of the work is done manually (HUIS 2013).

Subsequently to appropriate rearing conditions, mass rearing as well as further processing of insects requires the realisation of suitable and controllable approaches in processing units as well as measurement strategies. In this context, establishing and integrating non-invasive process monitoring systems is an essential prerequisite for the implementation of reliable process control strategies. During breeding, non-invasive measurement methods using camera measuring techniques and image processing algorithms, enable the real-time process monitoring by detecting pupae or even mealworm beetles e.g. in a breeding box. The detection of pupae facilitates the determination of an adequate time frame for reaping the insects, so that a machine vision-selection-system could be used to maintain the breeding of following mealworm generations. Related systems have already been established to remove for example grains with defects. Features like shadows, edges and colours are used to detect these damaged grains (PEARSON *et al.* 2012). As unhighlighted pupae in a box are hard to detect, the adaptation and application of concatenated histograms of local appearance features could serve as a method to detect pupae in a full box (LARIOS *et al.* 2008). The presented research adapts and applies these techniques for the rearing of insects in order to realise more efficient sorting and reaping processes to serve as a basis for the establishment of a robust and high-grade industrial rearing. Hence, we performed experiments with a monochrome smart-camera (Pictor T303-M-ETH, Vision and Control GmbH) in a lab scale rearing system. The detection of the objects and the extraction of information are realised by basic operations of the morphological image processing

such as erosion, dilution and thresholds. An Artificial Neuronal Network (ANN) was used to approach the classification task by finding connections between different features and categories in a potentially simpler way than by using rule-based programming. So, the advantage of an ANN is its ability of self-learning. One learning method, is the so called “supervised” learning, where the ANN is given a set of features, which have already been classified. The ANN is training itself to find a function with a minimized error by adjusting data processing parameters e.g. the weights of single information parts processed by a neuron. Thereto the pre-processed image data were further processed by a feed forward ANN from the neural network toolbox in MATLAB (R2016a, Mathworks). The ANN was fed with a pre-processed training data set consisting of 70 beatles, 300 larvae, 200 pupae and 60 dead larvae. The features used and tested for classification are circularity, grey level, fourier transformations, area, circumference and balance point. The application of the trained ANN showed an overall error rate of 2.6%. Specific error rates are 8.9% for beatles, 1.7% for pupae, 9.8% for dead larvae. Living larvae were entirely classified correctly. Comparisons with other classification methods e.g. decision trees, support vector machines revealed similar error rates of 2-5%. In order to additionally detect protein or fat composition of the larvae and the pupae, NIR-spectroscopic measurements are considered as supplement for the monitoring system. These parameters could contribute as further criteria for refining the ANN. Further, instead of single-sorting algorithms, pattern recognition algorithms for whole breeding boxes with clustered larvae are supposed be tested prospectively.

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Reproduction of *Tenebrio molitor* in an industrial production environment

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Industrial production of insects requires that individuals of all life stages are reared under conditions that are more intense than that of wild populations. This regards both high biomass yield and faster turnover during certain life stages (e.g. juveniles) as well as ensuring high performance of egg-laying adults that constitute the 'mother culture'.

In this presentation, we outline our recent work on the reproduction of *Tenebrio molitor*; including the impact of adult density, reproduction period (days in the same container) and age on reproduction. We include data from current work, but focus on a recently completed study comprised of two experiments: Experiment 1 examined the influence of four densities (0.11, 0.21, 0.42 and 0.84 beetles/cm^2) and five reproduction periods (1, 2, 3, 4 and 6 days) on reproduction. Experiment 2 examined the effects of age (30-92 days) and three densities (0.11, 0.21 and 0.32 beetles/cm^2) on reproduction. The total number of larvae per container was significantly affected by adult density and reproduction period, with the highest reproduction of 342 larvae at highest density (0.84 beetles/cm^2) and longest (6-day) reproduction period, and lowest reproduction of 18.6 larvae at the lowest density (0.11 beetles/cm^2) and the shortest (1-day) reproduction period. Daily per capita reproduction, however, was highest at the second lowest density (0.21 beetles/cm^2) and shortest reproduction period (1-day) with overall reproduction of 11.6 larvae/female/day, and lowest for the highest density (0.84 beetles/cm^2) and longest reproduction period (6-day) with an overall reproduction of 3.6 larvae/female/day. Age had a significant effect on reproduction both as the total number of larvae per container and number of larvae per female per day. The highest reproduction was found when beetles were 30 days old, but reproduction was in general highest for 13-29 days old females.

The highest density of beetles provides highest output of larvae. However, we do not consider the decrease in daily reproduction per capita as a sign of the beetles being stressed, but rather an adaption to the environment (i.e. high population density). Both adult beetles and larvae of *T. molitor* are observed to aggregate in dense groups in the production trays and are therefore not likely to be stressed due to high densities.

Mass-rearing of *Trichoplusia ni* larvae on semi-synthetic diet: effect on growth, development, reproductive competence and adult behavior

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Trichoplusia ni, an economically serious pest of agriculture, is relatively inexpensive to rear and its biology and behaviour have been extensively studied. In the last decade *T. ni* larvae have been infected with baculovirus to express a large number of proteins used for functional analyses and production of antigens for vaccine and diagnostic kits. The mass-rearing of insect larvae is therefore essential in a continuous supply of tests required to establish the best protocol of insect infection and production of antigens. Different artificial diets have been developed to facilitate the mass-rearing of insect but they can lead to reduced survival, lower fecundity and a longer developmental period. This study was focused on the modifications and improvements applicable to the diet and needed to enable a more successful rearing of *T. ni* beyond ten consecutive generations without any adverse effects on the insect's biological attributes. We evaluated the maintenance and propagation of a colony of *T.ni* reared at constant temperature, humidity and photoperiod using two different diets: the Diet 1 was the Modified MCmorran Grisdale commercial Diet, acquired from Great Lakes Forestry Centre, Sault Ste. Marie, Ontario; the Diet 2 was a homemade artificial diet originally proposed by SHOREY & HALE (1965) and modified with the addition of a mixture of vitamins, methyl paraben and linseed oil. The suitability of two diets was determined using an index incorporating growth, development, fecundity (indicated in terms of female pupal weight) and survival into one empirical factor, described by SETH & SHARMA (1998). Differences in larval period, female pupal weight and developmental period between diets were analysed using oneway ANOVA test if variances resulted comparable (Bartlett's test for equal variances) if not, the non parametric Kruskal-Wallis test was applied, $p<0.05$. The data obtained show that the Diet 2 seems to allow a shorter Larval Period of about 48 h with respect to Diet 1. All parameters compared resulted statistically different between the two diets, in particular the Larval Period resulted greater in Diet 1 than in Diet 2 ($p<0.05$), while the female pupal weight resulted significantly lesser in Diet 2 than in Diet 1. The Index of Adequacy for larvae that developed on the Diet 1 was about 6% lower than that for larvae that developed on the Diet 2. Growth indices revealed that the Diet 2 was better than Diet 1. The percentage of adult emergence was not very different, so both diets were efficient to guarantee the development of T.ni. The pupal characteristics were also important and useful for the assessment of the quality and vigour of T.ni, which included the calculation of the growth index and the evaluation of adult behaviour and mating competence of moths.

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Growth performance and nutrient contents of mealworms (*Tenebrio molitor*) fed with polystyrol

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Recent literature suggests using mealworms to dispose of polystyrol containing waste. It remains unclear, however, whether or not mealworms may use polystyrol as a nutritional source. Therefore a feeding experiment was conducted with a total of 6,000 mealworms (maggots of *Tenebrio molitor*), divided into twelve units of exactly 500 animals. They were kept in polycarbonate bowls in a controlled climate chamber for 28 days. Each 4 units were randomly allocated to one of three treatments (P, N, W), which differed in the feedstuff offered to the mealworms. Animals in treatment P received expanded polystyrol pieces. Animals in treatment N received no feed. Animals in treatment W received whole grain wheat flour. The number of animals alive, the biomass weight and the feed consumption of each unit were recorded weekly. After the trial, all mealworms were frozen and the contents of several nutrients were analyzed.

About 60% of all animals were lost during the experiment due to cannibalism irrespective of treatment.

Animals fed with wheat flour (treatment W) almost doubled their individual body weight from 71 mg/mealworm in the beginning to 136 mg/mealworm at the end of study. However, there was no gain in total biomass production due to the high amount of animal losses. Although polystyrol was obviously eaten by the mealworms (1.3 g per unit during the study), there was a loss of more than 50% of total biomass per unit, the same as for starving mealworms (treatment N). In contrast, mealworms of treatments P and N gained about 6 mg (8%) of individual body weight with a feed consumption of 22.5 g dry matter (DM) per unit.

The concentrations of nutrients did not differ significantly between the bodies of polystyrol fed mealworms and those of starving animals. In contrast, mealworms fed with wheat flour had significantly lower concentrations of dry matter (W: 27% vs. P: 31% and N: 35%) and crude protein (57% CP in DM vs. 73%) while concentrations of total fat (XL) were markedly higher (25% XL in DM vs. 12%). These results indicate, that mealworms of treatments P and N metabolized their energy storage of body fat to compensate nutritional energy deficiency.

In conclusion, mealworms may eat polystyrol, but they are unable to utilize the chemical bound energy for metabolism. For surviving, they degrade body fat stores. Therefore, polystyrol cannot be used as a feedstuff for growing mealworms.

Towards optimization of pre-slaughter starvation for black soldier fly larvae (*Hermetia illucens*)

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The use of black soldier fly larvae (*Hermetia illucens*) as alternative feed for livestock could help to decrease organic wastes by surcycling residual organic matter and provide novel animal feed ingredients Rearing, slaughtering and processing are crucial to maximizing their nutritional quality. Pre-slaughter starving periods use to evacuate fecal matter from the gut are variable and poorly documented. Indeed, a prolonged fasting can reduce the quality of larvae by inducing lipid mobilization. The gut evacuation time (GET) at 27°C of larvae varying in age and size, fed on Gainesville diet colored with carmine red? was determined by following feces excretion over 140 h. The impact of starvation time on crude lipids (CL: %DM) was measured on over time (96 h sampled at 0, 3, 6, 12, 24, 36, 48, 71, 96 h). The mean GET was 82.3 ± 2.1 h and was highly variable among indviduals (10 to 110 h). On a 96 h periods of starvation, CL and DM decreased about 2% and 1% respectively, showing the importance of starvation time on energetic mobilization. This project will contribute to identify an optimal pre-slaughter starvation time to reduce health risks by maintaining the quality of fly larval products.

Alteration of nutritional parameters of black soldier fly larvae by different feeding substrates

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Black soldier fly (*Hermetia illucens*) larvae are able to feed on any kind of organic material. More precisely, they are especially attracted to rotting organic material or manure. Such material is easily degraded by the larvae, why they play an important role for decomposition. Besides this, they are very potent in producing nutritionally valuable protein and, therefore, considered a part of the solution of the worlds' future food-supply problem.

Aside from being used as human food or animal feed black soldier fly larvae may also have an ecological impact due to their potential of reducing organic waste. To date a lot of organic waste accumulates over all stages of food production, i.e. at harvest, post-harvest, processing, distribution, and consumption stage. Conversion of such waste will also contribute to a more resource-efficient food production.

The aim of this project was to compare growth performance and nutritional parameters of black soldier fly larvae that were fed on different substrates, namely herbal waste, horse manure, residual minced meat, and leftovers of prepared meals. Small larvae (11 15 days after eclosion) were added to a polypropylene food-safe salad box filled to about one third with the respective substrate. Larvae were allowed to feed until first individuals began turning dark indicating transformation to prepupal stage. Substrate samples and larvae stored frozen after collection were analyzed according to methods of the official collection of analysis methods according to § 64 of the German Food and Feed Code.

We could observe differences in weight gain and growth depending on the fed substrate. Larvae were considerably smaller and lighter when fed on horse manure compared to all other substrates. Highest weight gain and growth was observed for larvae fed on prepared-meal leftovers. While the content of different carbohydrates (saccharose, glucose, fructose, lactose) in larvae did not reflect respective rates in substrates, this was the case for total fat content. Larvae fed on high-fat substrates also yielded higher fat contents (Pearson r = 0.88). However, for total protein contents this could not been observed.

In general, it can be concluded that the feeding substrate may have an impact on nutritional parameters. Although we could only show a correlation regarding the fat content, more in-depth analyses are needed on this subject.

Increasing the production output of common mealworm (*Tenebrio molitor*) using specifically designed feeds

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So far, common mealworm larvae produced for feed and food has often been produced on cheap and simple residues in the form of, for example, bran from various grains/cereals. However, research into the importance of the feed composition suggests that a diverse and varied feed with several different components provide a significantly higher output in the form of faster and increased larval growth. High quality feeds like commercial chicken feed mixes have been among the most effective, but also most expensive, choices for larval breeding. Yet, research into specific mealworm feed is causing the output volume to catch up (and overtake) that of the expensive chicken feed. Already now, these particular mixes result in dry mass outputs similar to commercial chicken feed and will most likely provide a higher output at a similar or lower cost after further adjustments. Like the dry feed component, preliminary experiments have shown that there is a large difference in overall output depending on the type of wet feed the larvae have available. Future research into both feed components will undoubtedly result in higher output volumes and potentially a much better quality of the final products.

In this presentation we will show some of the results that we have generated, at the Danish Technological during recent and ongoing R&D projects, on designing and developing specific feeds for *Tenebrio molitor*.

The impact of low temperature on the mealworm pupal stage - *Tenebrio molitor* Linnaeus, 1758 (Coleoptera, Tenebrionidae) – preliminary results for the use of pupae as a source of protein

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Abstract Entomophagy – a way of obtaining nutrients important for humans with minimal investment and minimal environmental burden is being brought into awareness of even gastronomically conservative parts of the world such as the countries of Europe. While around 1,900 species of insects are consumed worldwide (Africa, Central and South America, Asia or Australia), in Europe it is only a fraction. Insects can be legally consumed in the United Kingdom, France, the Netherlands and since 2015 also in Belgium. Switzerland has been trying to legalize the consumption of some insect species since 2015. In other European countries, insect consumption is rarer and promoted only by groups of people, who promote this way of life due to its sustainability, high nutritional value of insects, and low environmental impact compared to livestock farming.

Our research was focused on *Tenebrio molitor* Linnaeus, 1758 as a model species of edible insects. Mealworm larvae are commonly used as feed insects and as food for humans. There are many papers related to the consumption and the nutritional values of this developmental stage and the course of larval development. The consumption and development of mealworm pupae is generally less well known despite the fact that the pupae of some Lepidoptera or Hymenoptera are commonly consumed. The suitability of *T. molitor* pupae as a source of protein for human consumption is due to several reasons. Mealworm pupae have a demonstrably 7% higher total protein content than their larvae and about 4% lower fat content. Another important aspect is that the larvae empty their digestive system just before pupation. If we slow down the development of the pupae at an early stage, we will prevent the creation of the meconium (liquid waste of metamorphosis). Therefore, it is not necessary to "starve" the insects before consumption or further processing. In addition, less sclerotisation of the early pupal stages compared to larval stages makes mechanical processing or further treatment by the consumers easier. The know-how of keeping the individuals in pupal stage, without physiological process disruption, is important for the creation of suitable conditions for mass production.

This research builds on work from 2016, when the effect of different temperatures on the length of *T. molitor's* pupal development under laboratory conditions was studied. In the present study, we observed the development of pupae after suppression of metamorphosis at 4°C and subsequent restart at an optimum temperature of 28°C.

Altogether 2,900 pupae were divided to 116 Petri dishes each containing 25 fresh pupae. After 15 to 76 days of exposure to low temperature, 50 pupae (two Petri dishes) were displaced to a temperature of 28°C. The temperatures from 25°C to 30°C seems optimal for the development of this species. The control group consisted of 2,500 pupae divided to 100 Petri dishes containing 25 fresh pupae incubated at 28°C. Low temperature impact on the timing of adult hatching and hatching success was evaluated. T-test was used to determine the significant difference between hatching success values in relation to the length of exposure to low temperatures. In the second step, the data were integrated to five day intervals and an ANOVA was used for statistical comparison.

The development of the pupae stagnated at 4°C. Adults began to hatch 6.47±0.95 days after moving to the optimal temperature of 28°C. All adults hatched at 4-14 day interval (7.72 days on average) and 91.86% of adults hatched out in three days (7th - 9th day). The time until the beginning of the adult hatching was shortened by 0.57 days compared to the control group (7.04±0.71 days). Average time of hatching was extended for 0.15 days (7.57 in control group) and the hatching interval was extended by seven days (6 - 9 days in the control group).

The average hatchability (95.10%) after transfer to optimal conditions was approximately 4% lower compared to the control group (98.96%). Viable individuals represented 97.86% of hatched individuals. All individuals hatched in interval 5 - 10 days were viable. Viable individuals hatched on the eleventh day represented 89.47%. All individuals hatched outside the range of 5-11 days were damaged.

There was a significant difference (t–test; $p<0.001$) in the hatching success in relation to the length of exposure to low temperatures. In the case of integrated data to five day intervals there was no significant difference (ANOVA – $F=1.44$; $p=0.347$, Kruskal-Wallis – $p=0.2618$). In both cases, was not possible to determine the trend of increasing or decreasing hatchability in relation to the length of exposure.

Based on the small difference in the duration of the pupal stage of the control specimens and specimens exposed to low temperatures, followed by optimization of the temperature, it is possible to assume that the development of pupae can be slowed to a minimum or paused completely, while maintaining physiological processes.

However, it should be noted that there are losses (although minimal) caused by this procedure. These represented about four percent compared to continuous pupal development under optimal conditions.

Key words: Mealworms, entomophagy, pupa, temperature, *Tenebrio molitor*

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Lab-scale production system for tailored rearing of house crickets: Experimental evaluation and perspective

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In the recent years, it has been spread the idea that insect rearing can offer a valid alternative to livestock industry, in terms of ecological, financial and social impact. To the current day, this perception is surrounded by controversial opinions, mostly due to the different performances spotted in the literature.

In order to correctly assess the potentiality of house cricket (*Acheta domesticus*), it is required not only to evaluate their behavior into a scale production simulating system, it is also necessary to observe and optimize all the parameters that affect the final yield, such as population density, feeding acceptance, conversion efficiency and instar discrepancy.

In a first step a tailored rearing system was designed to investigate the performances of house crickets. The rearing system consists of two climatic chambers to control environmental parameters (T, RH, light cycles), eight tailor made rearing boxes constructed to fit all the insects needs in terms of hidden spaces and clean environment, while minimizing the required manual operations. All the equipment in direct contact with the insects is autoclavable and allows to perform the experiments in a controlled and statistical-friendly manner.

In a second step the work aims to monitor, evaluate and optimize technological treatments on cricket eggs, in order to synchronize hatching, therefore reduce cannibalism episodes or the stressful and expensive human intervention to separate differently aged groups. Different treatments as a_W of the substrate, temperature of storage, time/temperature treatment are assessed and optimized using Central Composite Design system, for the purpose to find a set of combination economically sustainable, both in terms of hatching rate and financial effort of the operator. Differences on the chemical composition of the individuals that came out from treated eggs will be evaluated in further studies and non-treated parents will be set as control.

Hermetia illucens defatted larvae meal as partial dietary protein source for rainbow trout (*Oncorhynchus mykiss*): intestinal microbial communities and hepatic oxidative stress

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Scientific effort is being done on replacing fishmeal (FM) with more sustainable dietary protein sources in fish feeds. Insect meals have been reported to have a good nutritional composition and to be a promising sustainable alternative protein source. Moreover, insects grow quickly on low-quality organic waste, do not require arable land and present a high feed conversion efficiency. Recent studies have been demonstrating the impact of diet composition on intestinal bacterial communities, which, in turn, may modulate health and well-being status of fish. Therefore, the purpose of this study was to investigate the effects of dietary inclusion of *Hermetia illucens* (Diptera: Stratiomydae) defatted larvae meal (Hi) as partial replacer of FM on rainbow trout (*Oncorhynchus mykiss*) performance, hepatic oxidative stress and intestinal mucosa- (MAB) and digesta-associated bacterial communities (DAB). Three isoproteic (about 45% crude protein), isolipidic (about 15% crude lipids) and isoenergetic (about 22MJ/kg d.m.) experimental diets were formulated with FM as main protein source (Hi0) or substituting 25% or 50% of FM with Hi (Hi25 and Hi50, respectively). The Hi was purchased from Hermetia Baruth GmbH (An der Birkenpfuhlheide Baruth/Mark, Germany). Rainbow trout (initial average body weight: 178.9 ± 9.81 g, mean ± SEM) were randomly allocated to four replicate tanks per dietary group and fed each experimental diet (daily feeding rate corresponding to 1.5% of body weight), for 11 weeks. At the end of the growth trial, fillet yield was recorded for 30 fish per dietary group, while organosomatic parameters were determined in six fish per dietary group. The antioxidant enzymes involved in the redox potential of fish, catalase, glutathione peroxidase, glutathione reductase, superoxide dismutase and glucose 6-phosphate dehydrogenase, as well as lipid peroxidation, were also determined in 10 livers per group. To study the MAB and the DAB, denaturing gradient gel electrophoresis (DGGE) was performed on six intestine tissues and six intestine contents per dietary group, respectively. To get an overview of the complex communities, richness and biodiversity parameters were calculated on the DGGE banding patterns and ANOSIM was performed to determine the extent of the differences between the three dietary groups in the MAB and in the DAB. To identify dominant bacte-

rial groups, as well as suggesting to which functions the sequenced strains could have on fish, selected bands were excised from the DGGE gel, sequenced and deposited in the GenBank database. The Web-based BLASTN tool was used to find closely related nucleotide sequences; to increase the accuracy of the assignments, different sequence similarity thresholds were used for different taxonomic levels: a similarity ≥97% for species level identification and 95%, 90%, 85%, 80% and 75% for assignment at the *genus*, family, order, class and *phylum* level, respectively.

Growth performances, fillet yield and organosomatic parameters were similar among the dietary groups. Similarly, specific activity of antioxidant enzymes in rainbow trout liver was not affected by the dietary treatment, while hepatic lipid peroxidation level decreased with the increase of dietary *H. illucens* level in the diet ($p<0.01$). Dietary inclusion of Hi affected the structure and the composition of the both MAB and DAB communities. The biodiversity of the MAB Hi25 group was significantly higher than that of the control group ($p<0.05$) and the Hi50 group stayed in an intermediate position between the control and Hi25 groups. As showed in **table 1**, the results of the pairwise comparisons showed that differences between all the groups were significant ($p<0.01$) in the DAB, while in the MAB only the pair Hi0-Hi25 displayed a significant difference ($p<0.05$). Irrespective of the diet, the sequencing of the excised bands highlighted a clear prevalence of γ-Proteobacteria, though α-, β-Proteobacteria and Actinobacteria were also present in the MAB of fish fed Hi-based diets; the DAB of the fish fed Hi-based diets presented a clear increase in bacteria belonging to the Firmicutes *phylum*.

Table 1: ANOSIM results of the pairwise comparisons between each treatment.

		MAB		DAB	
		Hi0	**Hi25**	**Hi0**	**Hi25**
ANOSIM	**Hi25**	0.3861*		0.3500**	
R values	**Hi50**	0.1343	0.1176	0.5222**	0.5074**

*** p-values<0.01; * p-value<0.05.*

The global analysis of our preliminary results suggests that *H. illucens* defatted larvae meal is a valid alternative protein source to FM for rainbow trout feeding. Up to 50% of FM may be replaced by Hi without affecting growth performance, hepatic liver oxidative status, while liver peroxidation is inclusively reduced. The results of the microbiological assays revealed that also the intestinal bacterial communities were sensitive to dietary changes, having shown an increased community biodiversity in the Hi-fed groups. Several OTUs retrieved could be accountable for effects on fish physiology, going from probiotic bacteria, to bacteria able to produce eicosapentaenoic acid, to those able to hydrolyse carbohydrates. Some bacteria may protect fish from pathogens as well as enhance digestion mechanisms, physiological functions and welfare in general. The outcome of this study may benefit future research into both the microbial community of Salmonids and into the interaction between host, guest and diet. To delve into the biological consequences of the host-guest interaction, we encourage to combine the description of the bacterial community with functional analyses, such as metagenomics, metabolomics, challenge tests and alike.

Keywords: rainbow trout, insect, Black Soldier Fly, intestinal microbial community, DGGE, sustainable aquaculture.

Total substitution of soybean protein source with *Hermetia illucens* meal in Lohmann Brown Classic hens diet: effect on liver and visceral fat composition

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At present, soybean is the main protein source in poultry feed but the need for alternative ingredients has been growing due to the continuous increase of prices, and for environmental aspects. In this context, insects are considered a viable attractive solution for animal feeding, especially for poultry since they are natural food sources for them. Despite the high attention of the international scientific community on the possible utilisation of insect meal, the information about the effect of the substitution of soybean with insect meal on the characteristics of tissues such as liver and visceral fat is scarcely investigated. The present study aims to fit this gap by evaluating the effects of a total replacement of soybean with *Hermetia illucens* defatted meal as protein source on physical characteristics, lipid content and composition of both liver and visceral fat. Twenty Lohmann Brown Classic laying hens were farmed from 24 to 45 weeks of age and divided into 2 groups fed two isoproteic and isoenergetic diets, differing in the main protein source: soybean meal based diet (SB group) or *Hermetia illucens* larvae defatted meal based diet (HI group). At the end of the trial, hens were sacrificed and both liver and visceral fat were collected. Physical characteristics, as weight, colour parameters (lightness - L*, redness index - a*, and yellowness index - b*), and pH were recorded, and Chrome, Hue, Colour difference (ΔE) were calculated from colour parameters. In addition, the lipid content and fatty acid composition of liver and fat were analysed by gas-chromatography in order to verify if and how they were affected by the diets. ANOVA analysis of data was conducted for detecting the statistical differences among the two experimental groups.

From physical analyses emerged that livers from the two different experimental groups significantly differed in lightness (L*) and yellowness index (b*), as well as in the calculated values of Chrome and Hue. Specifically, HI group showed liver higher in L* values (37.17 vs 31.84) and more yellow (17.70 vs 12.09) with difference observable by the eyes of non expert people (ΔE>5). Weight and pH were not significantly different. In parallel, colour parameters differed ($p<0.05$) in fat of HI and SB groups, HI group presenting fat with lower L* value (60.95 vs 64.55) and higher b* value (21.09 vs 15.23) than fat from SB group, thus resulting in a visible colour difference (ΔE>5).

Regarding lipid composition of liver (**Table 1**), diets affected both total lipid content and its fatty acid composition ($p<0.05$). HI hens presented liver higher in fat than SB group.

As a consequence, total fatty acids increased in HI liver. The analysis of fatty acid composition revealed that C18:1-n9cis, C16:0, C18:2-n6cis, and C18:0 were the main fatty acids, amounting at 55% of the global fatty acid profile. HI liver presented lower contents of C18:1-n7 and C20:4-n6 than SB liver, however no significant differences in saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) of both n6 and n3 series were found.

Concerning the visceral fat composition, C18:1-n9, C16:0, and C18:2-n6cis supplied the 65% of total fatty acids. HI visceral fat resulted in a lower content of C16:0, C16:1-n7, and C18:1n7 than SB group visceral fat. On the contrary, C18:0 and C18:2-n6 were found significantly ($p<0.05$) higher in HI visceral fat. Globally, these differences lead the significant lower MUFA and PUFA-n3 contents and higher PUFA-n6 values found for HI fat than for SB one.

In conclusion, a total substitution of soybean with *Hermetia illucens* larvae meal as protein source of the diet for Lohmann Brown Classic hens significantly changed the colour of both liver and visceral fat. Moreover, diet affected liver lipid content without alteration of its fatty acid composition. On the contrary, total lipid content of visceral fat was not affected by the inclusion of insect meal in the diet whilst its fatty acid composition was deeply modified.

Table 1: Total lipids (g/100g tissue), total fatty acids (TFA, g/100g total lipid), and fatty acid profile (g FA/100g TFA) of liver and visceral fat from Lohmann Brown Classic Laying hens fed with soybean meal based diet (SB) and *Hermetia illucens* meal based diet (HI).

	Liver				Visceral fat			
	Diet		Sign.	MSE	Diet		Sign.	MSE
	SB	HI			SB	HI		
Total lipids	5.76b	7.38a	< 0.05	1.357	70.01	66.12	ns	7.563
Total fatty acids	5.22b	6.98a	< 0.05	1.446	70.70	71.28	ns	7.286
C12:0	1.24	1.32	ns	0.071	1.33b	2.22a	< 0.05	0.494
C14:0	1.66b	1.94a	< 0.0001	0.075	1.85b	2.23a	< 0.05	0.241
C16:0	18.03	17.96	ns	0.801	19.63a	17.83b	< 0.05	1.298
C16:1-n9	1.14b	1.27a	< 0.05	0.085	1.14	1.12	ns	0.052
C16:1-n7	2.32	2.13	ns	0.354	3.64a	2.86b	< 0.05	0.428
C18:0	9.92	10.42	ns	0.885	5.50b	6.11a	< 0.05	0.521
C18:1-n9cis	24.37	26.70	ns	2.747	31.04	30.65	ns	0.703
C18:1-n7	3.58a	3.30b	< 0.05	0.120	3.81a	3.45b	< 0.05	0.129
C18:2-n6cis	10.41	10.88	ns	0.986	16.53b	18.64a	< 0.05	1.549
C20:0	1.19	1.16	ns	0.058	1.08	1.05	ns	0.042
C20:3-n6	1.03a	0.91b	< 0.05	0.103	0.60a	0.56b	< 0.05	0.020
C20:4-n6	7.24a	5.65b	< 0.05	1.276	0.60	0.59	ns	0.025
C22:5-n6	1.62a	0.86b	0.0001	0.307	0.05	0.06	ns	0.018
C22:6-n3	2.44	2.12	ns	0.484	0.46a	0.44b	< 0.05	0.015
SFA	35.81	36.47	ns	0.686	32.85	32.75	ns	1.041
MUFA	34.44	36.38	ns	3.035	42.51a	40.88b	< 0.05	0.943
PUFA-n6	22.29	20.17	ns	2.198	19.16b	21.18a	< 0.05	1.541
PUFA-n3	5.94	5.49	ns	0.578	4.09a	3.88b	< 0.05	0.140

Sign.: Significance;
ns: not significant.
The fatty acids C13:0, C14:1-n5, C15:0, C16:1-n9, anteiso-C17:0, C17:0, C16:3-n4, C17:1, C18:3-n6, C18:3-n4, C18:4-n3, C20:1-n11, C20:1-n9, C20:2-n6, C20:3-n6, C20:4-n6, C20:3-n3, C20:5-n3, C22:0, and C22:6-n3, found in quantity below 1 g/100 g total fatty acids, were detected and considered in the fatty acid groups but not reported in the table for brevity.

Black soldier fly larval meal demonstrates high levels of anti-protease activity in rainbow trout and Nile tilapia intestinal homogenates

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High inclusion levels of insect meal in fish diets have been reported to reduce growth performance. This was generally attributed to the presence of chitin that could reduce protein digestibility. However, anti-nutritional factors such as protease inhibitors present in insect meal could also intervene. The present study aims to characterize the in vitro effects of increasing levels of insect meal on the digestive protease activity of rainbow trout and tilapia. According to MOYANO *et al.* (1999), 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 meal was obtained from black soldier fly larvae fed on a Gainesville diet for 5, 10, 15, 20, 25 and 30 days. Larvae were analyzed for energy, dry matter, ash, fat, protein and chitin using standard methods. Anti-protease activity (expressed as the % of inhibition/ug of inhibitor per UA enzyme activity) was found to vary as a function of larval stages and fish species. The protease inhibition was higher with meal produced from larvae at early-stages of development (5, 10 and 15 days) for both fish species. Moreover, protease inhibition was higher in tilapia than in rainbow trout (51.1 1.9% and 19.8 0.4% for 30 days-old larvae at 300 ug/UA, respectively). The anti-proteases in black soldier larvae still need to be identified and characterized. 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.

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Nutrient digestibility in laying hens fed *Hermetia illucens* larvae meal from 24 to 45 weeks of age

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In the last few years there has been a population increase and it is estimated that the world population could be reach 9 billion in about 30 years (FAO 2013). This will result in an increase in demand for food of animal origin (meat, fish, milk and eggs) and consequently an increase in the demand for protein sources. The conventional protein sources are soybean and fish meals, but rising prices and increasing demand will not accommodate all the request (FAO 2013); for this reason, researchers are interested to study alternative protein sources not only in humans, but also in pet and farming animals. Recent studies have shown that insects can be a valid protein source in poultry. In fact, the main problem in the use of insect as animal feed is the chitin content. Chitin is the major component of the insect exoskeleton able to negatively affects protein digestibility (LONGVAH *et al.* 2011). The chickens normally eat worms found in the soil and can synthesize chitinases, enzymes that hydrolyze chitin (BOKONYI & GAL 2010).Very few studies are available in literature on the use of insect meals in laying hens and the results are conflicting. MAURER *et al.* (2016) showed that *Hermetia illucens* larvae meal included up to 100% on protein basis in a laying hens diet did not affect egg production, feed intake and feed conversion efficiency. MARONO *et al.* (2017) evaluated the effects of a full replacement of soybean meal with a defatted meal from *Hermetia illucens* and their results showed a decrease of feed intake and consequently of productive performance. So, further investigation needs to improve the knowledge on this topic. The aim of this study was to estimate the nutrient digestibility of laying hens fed *Hermetia illucens* larvae meal as a complete replacement of soybean meal from 24 to 45 weeks of age. The trial was carried out on a private farm near Caserta (Southern Italy). A total of 108, 24 weeks old Lohman Brown Classic laying hens (average body weight 1.78 kg ± 0.15) were divided into 2 groups (54 hens per group). The hens, housed in the same building under controlled temperature and humidity condition, were allocated in modified cages (800 cm^2/hen). For each group, the birds were divided in 3 cages (18hens/cage); each cage was divided in 3 equal areas, to obtain 9 replicates of 6 hens per group. The dark: light cycle was 9:15 h. The feed and the water were distributed by hand *ad libitum*. The groups were fed two isoprotein and isoenergetic diets, formulated to meet hens requirements (Lohmann Brown classic Management Guide, 2011), differing for the ingredient used as protein source: the control group fed a corn-soybean meal based diet (SBM), while in the other group (HILM) the soybean meal was completely replaced by

Hermetia illucens larvae meal (Hermetia Deutschland GmbH & Co KG, Amtsgericht Potsdam, DE).

Table 1: Chemical nutritional characteristics of *Hermetia illucens* larvae meal diet (HILM) and Soybean meal diet (SBM).

	***Hermetia illucens* larvae meal diet (HILM)**	**Soybean meal diet (SBM)**
Chemical nutritional characteristics		
DM, %	90.5	90.1
CP, % as fed	17.9	18.1
Crude fibre, % as fed	4.14	3.96
Ether extract, % as fed	4.26	4.33
ADF, % as fed	3.82	3.45
ADIP, % as fed	2.88	1.52
Ash, % as fed	14.2	14.2
NDF, % as fed	15.2	14.0
ME, kcal/kg	2,745	2,780

Ingredient(g/kg) HILM diet:maiz grain 653.0, Insect meal, CaCO3 grains 80.0, Dehulled sunflowers meal 50.0, Vegetable oil: 10.0, MinVit 30.0, Monocalcium Phosphate 5.00, Salt 2.00.**Ingredient(g/kg) SBM diet**:maiz grain 583.0, Soybean meal 235.0, $CaCO_3$ grains 80.0, Dehulled sunflowers meal 50.0, Vegetable oil: 15.0, MinVit 30.0, Monocalcium Phosphate 5.00, Salt 2.00.Provided 20 g of celite and 10 g of mineral and vitamin supplements. Per kilogram: vitamin A (retinyl acetate) 20,000 IU, vitamin D3 (cholecalciferol) 6,000 IU, vitamin E (dl-α-tocopheryl acetate) 80 IU, vitamin B1(thiamine monophosphate) 3 mg, vitamin B2 (riboflavin) 12 mg, vitamin B6 (pyridoxine hydrochloride) 8 mg, vitamin B12 (cyanocobalamin) 0.04 mg, vitamin K3 (menadione) 4.8 mg; vitamin H (d biotin) 0.2 mg, vitamin PP (nicotinic acid) 48 mg, folic acid 2 mg, calcium pantothenate 20 mg, manganous oxide 200 mg, ferrous carbonate 80 mg, cupric sulphate pentahydrate 20 mg, zinc oxide 120 mg, basic carbonate monohydrate 0.4 mg, anhydrous calcium iodate 2 mg, sodium selenite 0.4 mg, choline cloride 800 mg, 4-6-phitase 1,800 FYT, D.L. methionine 2,600 mg, canthaxanthin 8 mg**.**

The coefficients of ileal apparent digestibility were measured using the indirect method of the AIA (VOGTMANN *et al.* 1975). Celite has been added at 2% to the diets as an indigestible marker. At 45 weeks of age the hens were slaughtered, the illeum was separated from the intestine (20 mm after Meckel's diverticulum to 40 mm proximal to the ileocecal junction) and the digesta were pooled per replicate, freeze-dried and ground to pass a 1mm sieve and stored at -20°C until analysis. Chemical composition of the diets were (**Table 1**) and of ileal contents were determined according to AOAC (2004). The apparent ileal digestibility coefficients of dry matter (DM), organic matter (OM), crude protein (CP) and ether extract (EE) were measured as follows: 100 – 100 × [(% AIA in the diet/% AIA in the ileal content) × (% nutrient in the ileal content/% nutrient in the diet)].The results were processed by one- way ANOVA (SAS, 2000). Comparison between means was performed by Tukey's test.

Table 2: Coefficients of apparent ileal digestibility of hens(%)

	HIML	SBM	RMSE	P value
Dry matter	67.63b	70.27a	2.36	0.0421
Organic matter	69.17b	72.18a	2.24	0.0177
Protein	67.67B	78.29A	1.84	<0.0001
Lipid	73.01	77.34	5.03	0.1071

Abbreviations: HILM: *Hermetia illucens* larvae meal; SBM: Soybean meal; RMSE: root mean square error. A, B: P value <0.05; a,b: P value <0.001.

The reduced coefficients of apparent ileal digestibility of dry and organic matter in layers fed HIML diet (- 4.3 and -4.2% than SBM group, respectively) were mainly due to the strong reduction of protein digestibility (-13.6%). Even if not significant, also ether extract digestibility was decreased by 5.6% in comparison to SBM group. These results are in line with other findings available in literature. KROECKEL *et al.* (2012) observed that turbots (*Psetta maxima*) fed *Hermetia illucens* pre-pupae meal (30% of inclusion in the diet) showed a lower digestibility for organic matter, crude protein, crude lipid and gross energy than the control diet. SCHIAVONE *et al.* (2014) and BOVERA *et al.* (2016) described a decreasing of protein digestibility in broilers fed *Tenebrio molitor* larvae meal in comparison to a control diet. PICCOLO *et al.* (2017) demonstrated that the inclusion at 25% of *Tenebrio molitor* larvae meal in the diet for gilthead sea bream(*Sparus Aurata*) not showed any negative effect on digestibility of crude protein and ether extract; instead it was possible observe a decreasing of the apparent digestibility coefficients on crude protein and ether extract in gilthead sea bream fed *Tenebrio molitor* (50% of inclusion in the diet).The decreased nutrient digestibility observed in this trial can be attributed to the presence of chitin in the insect meal.

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Tenebrio molitor, *Acheta domesticus* and *Ruspolia differens*: powder sensory evaluation and fraction characteristics

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According FAO report, there are more than 1900 known species of edible insects (ARNOLD VAN HUIS 2013). Insects are an important food source for humans, and references to their nutritional value are found in several articles across a range of scientific disciplines and have been reported to provide essential nutrients to the human diet (DEFOLIART 1995, 1999, GLADYS O. LATUNDE-DADA 2016). Therefore, supplementing bakery and confectionery products with insect powder would considerably increase the nutritional value of product.

The taste of insects depends on many factors – primarily the species of insects and stage of life-cycle (MLCEK *et al.* 2014). Many common edible insect species has specific taste that reminds crustaceans like shrimps, etc. When served right, it may have an advantage for the whole experience and especially in sour recipes, where it may even help to enhance desired taste. On the contrary, the taste can become a limiting factor, when there is a need to produce confectionery or bakery products.

This research was carried out to find most suitable dehydration methods to make insect powder for bakeries and confectionery industry. The aim was to select a method that allows to produce a powder, that could supplement cereal flours without complementing taste and flavor.

The influence of two dehydration methods on sensory properties of three species of edible insects, mealworms (*Tenebrio molitor*) and crickets (*Acheta domesticus*), grasshoppers (*Ruspolia differens*), was determined, using standard methods: oven-drying (temp. 1600 C for 90 min.) and freeze-drying (36 hours). Depending on method, the insects were grinned into powder or paste form before or after dehydration process and dehydrated until the same moisture content (+/-2%).

Sensory analysis was carried out for all six insect powder samples using Descriptive Analysis method. Overall likeness, intensity, aroma and taste were described. Thus, it was found that freeze-dried samples had weaker aroma and taste profile, but stronger seafood flavor, compared to oven-dried samples. Therefore oven-dried powder could have benefits for bakery and confectionery products, based on their sensory characteristics.

Fractions were studied with bright-field microscopy, to determine insect body parts in different fractions. Fractions were crushed with grinder and mortar and differences in fractions were described.

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Inactivation of natural contaminating microorganisms and *E. coli* O157:H7 in black soldier fly larvae by high hydrostatic pressure treatments

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Trends predict a steady increase in population to reach nine billion people in 2050 forcing to an increase in the production of food and feed, which can affect the agricultural ecosystems resulting in an even greater pressure on the environment than the current one (VAN HUIS *et al.* 2013). The consequence could be a shortage of land for cultivation, water, forests, fisheries, biodiversity resources as well as nutrients and non-renewable energy (VAN HUIS *et al.* 2013). The above predictions center the focus on insects as one of the potential sources of protein for animal or human consumption. The use of edible insects as a sustainable protein supply has been recently promoted by the FAO because edible insects have always been part of the human diet accounting for about 2,000 edible species (VAN HUIS *et al.* 2013). Insects are a potential source for conventional production (mini-livestock) of protein, either for direct human consumption or indirectly in new foods made from insect protein; and as a protein source into feedstock mixture (HALLORAN *et al.* 2016).

Hermetia illucens is a very efficient organism that can be used in the management of organic waste (SHEPPARD *et al.* 1994). The pre pupa can be used in animal feed because it has a high fat and protein content (30% and 40% respectively) (ST-HILAIRE *et al.* 2007). However, one of the main concerns of the feeding system is the hygiene of pre pupae and compost produced (LALANDER *et al.* 2014).

High hydrostatic pressure (HPP) is one of the most popular non-thermal preservation technologies. The effect of high-pressure treatment has been demonstrated to be capable of inactivating *E. coli* in beef meats (LI & GÄNZLE 2016), poultry meat (RENDUELES *et al.* 2011), vegetables (VAN OPSTAL *et al.* 2005), ovine milk (GERVILLA *et al.* 1997), fruits (BAYINDIRLI *et al.* 2006), seafood (MENGDEN *et al.* 2015).

The present work focuses on inactivating natural contaminating microorganisms and inoculated *E. coli* O157:H7 in black soldier larvae, in order to assess the usefulness of high hydrostatic pressure in controlling pathogenic microorganisms that potentially can contaminate the larvae. Additionally, the best model describing inactivation curves for *E. coli* O157:H7 was studied. GInaFiT software (GEERAERD *et al.* 2005) was used to fit survival data to different mathematical models. The mathematical models contained in the GinaFit software used in this study were: Weibull model (MAFART *et al.* 2002, GEERAERD *et al.* 2005), the Cerf model with shoulder (Biphasic model) (CERF 1977) and the Log-linear model (GEERAERD *et al.* 2000).

Results indicated that high hydrostatic pressure was effective against natural contaminating molds and yeasts. The logarithm of survivor of yeasts and molds showed a reduction close to 3.03 log cycles by applying a pressure of 250 MPa for 15 min. At the same conditions, the reduction achieved for aerobic mesophilic bacteria counts was only about 0.12 log cycles. In the current study, no surviving yeast and mold were found in the larval samples by using a pressure of 400 MPa for 2.5 min at 25°C, while only 0.35 log reductions were achieved for total aerobic mesophilic microorganisms. As for *E. coli* O157:H7, the inactivation level increased as the pressure increased. The treatment at 350 MPa for 10 min promoted a reduction of 3.93 log cycles and of 2.78 log10 CFU/g with 400 MPa for 1 min. The maximum reduction level was achieved at 400 MPa for 7 min. reaching more than 5 log cycles reductions. The inactivation curves for the studied pressures (250, 300 and 400 MPa) never followed a straight line; thus they could not be analyzed by using a log-lineal model as the Bigelow model. The model that better described the inactivation pattern of the cells among those studied was the Biphasic model with the lower Akaike index.

The results in the study showed that high hydrostatic pressure is an excellent method to hygienize black soldier larva at the same time that inactivation the pathogenic bacteria studied.

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"Better safe than sorry": identification of allergic risks of consuming edible insects through a systematic review

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The expected future demand for food and animal-derived protein will require environment-friendly novel food sources with high nutritional value. Insects may be one of such novel food sources. However, there needs to be an assessment of the risks associated with their consumption, including allergic risks. Regarding insect consumption the allergenic risk may arise due to cross-reactivity with known allergic sources such as house dust mites and crustaceans. Therefore, a systematic review was performed aiming to analyse current data available regarding the allergic risks of consuming insects. In addition to the search in databases (PubMed/Medline, Scopus and Web of Science) the authors also considered previously known studies for inclusion in the review. All reported cases of food allergy to insects were reviewed, and the possibility of cross-reactivity and co-sensitisation between edible insects, crustaceans and house dust mites was also studied. A total of 25 articles – 8 assessing the cross-reactivity/co-sensitisation between edible insects, crustaceans and house dust mite; 3 characterizing allergens in edible insects and 14 case reports, case series or prevalence studies of food allergy caused by insects were included in the review and analysed. Cross-reactivity/co-sensitisation between edible insects and crustaceans seems to be clinically relevant as seen in studies performing double-blind placebo-controlled food challenge (DCPCFC) with shrimp-allergic patients. This hypothesis is further supported by case reports where subjects who developed food allergy to insect consumption had previous history of food allergy to crustaceans. Although there are studies showing that extracts from edible insects can IgE-bind to sera from house dust mites-allergic patients it is still unknown if co-sensitisation between house dust mites and edible insects can lead to food allergy. This is particularly concerning because if cross-reactivity between house dust mites and edible insects has clinical significance, the use of edible insects on the food industry could be in jeopardy due to the high rate of house dust mite-sensitised individuals. It also seems that individuals who are constantly exposed to edible insects (e.g. insect-rearing workers) may develop both inhalant and food allergy to the edible insect. Additionally, more information is also needed about the molecular mechanisms underlying food allergy to insects, although current data suggest that an important role is played by ar-

thropod pan-allergens such as tropomyosin or arginine kinase. More studies performed with individuals primarily sensitised to edible insects because the allergens responsible for cross-reactivity may be different from those involved in food allergy in individuals only sensitised to the edible insects.

Technological and functional characterization of cricket powder for new food product development

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The interest for insects as alternative protein sources is becoming bigger everyday as a consequence of increasing world population and demand for natural resources. Such novel ingredients should be approached with a multifaceted aspect. Technological properties of *Gyllodes sigillatus*, also called “The banded cricket” powder were tested and compared with wheat flour to evaluate the possibility to use it as a new ingredient in product development. Solubility of cricket powder was found 21% and water absorption capacity was measured as 101.2%; these values were higher than wheat flour. Emulsion capacity of cricket powder (10.5%) was lower than wheat flour (33.8%) however greater emulsion stability occurred with cricket powder (84.7%) than with wheat flour (50%). Similar results were obtained for the foaming properties. Foaming capacity of cricket powder was 28% lower than wheat flour despite a higher stability of foam (<85%) compared to wheat flour (≥75%). Cricket crackers were developed by replacing 30% of wheat flour. Results of survey suggested that those who are open to trying novel protein sources are also willing to adopt insects in their daily diets. Sensory analysis results showed that the crispiness was the most liked attribute of the insect-enriched crackers while the color and the taste were not appreciated by the panellists. GI of the cricket crackers was estimated at 62.3 which classifies this product among the Intermediate GI foods.

Could *Galleria Mellonella* (Lepidoptera Pyralidae) be a source of n-3 fatty acids in human diet?

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Lipids play major roles in the insect life and are involved in multiple metabolic functions; in the same time insect lipids represent also a potential source of essential fatty acids known to exert beneficial effects in human health. The waxmoth *Galleria mellonella* showed an n-3 polyunsaturated acid content mostly dependent by feeding substrates and interesting increase in its value was reached with n-3 source supplements to diets. Due to the possibility to affect n-3 fatty acid content by diets, *G. mellonella* larvae were reared on a standard diet (D1) and on the same diet enriched with linseed (D1+), in order to obtain n-3 fatty acid value and a n-6/n3 ratio that meet requirements for human health, in particular in the prevention of cardiovascular diseases.

The most representative fatty acids in *G. mellonella* larvae from both D1 and D1**+** diets, resulted palmitic acid among SFA, oleic acid among MUFA and α-linoleic among PUFA. The larvae reared on (D1**+**) diet showed a significant decrease of palmitic acid (SFA), as well as palmitoleic and heptadecenoic acid levels (MUFA) and as result a lowering of total SFA and MUFA. Diversely, the supplement of linseeds determined a significantly increase of stearic acid (SFA), α-linoleic and α-linolenic acid (PUFA). Thus, larvae fed on D1**+** showed a higher PUFA content and a lower SFA/UFA ratio than those reared on D1 diet. In addition, n-6/n-3 ratio was lower in larvae fed on D1**+** (0.85) than those reared on D1 (4.68).

The addition of a natural source of n-3 fatty acid to *G. mellonella* feeding substrate resulted to be effective in improving fatty acid composition of this insect, making it suitable to human consumption as a potential natural source of unsaturated fatty acids (UFA) and especially of n-3 fatty acids.

Determination of vitamin B12 in mealworm (*Tenebrio molitor* larvae) using preparative immune-affinity chromatography and ultra-high performance liquid chromatography

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Insects are highly nutritious, not only as a part of traditional diets, but also as a novel food. They are especially rich in protein and can surpass other foods of animal origin. Besides high protein content, they are also rich in minor nutrients such as vitamins. Although the total amount of vitamin B12 daily needed is rather low (app. 3 µg), matching the daily demand is still challenging, as vitamin B12 is mostly present in foodstuff from animal origin. Vitamin B12 malnutrition is thus very common in the third world as well as among people living on a vegan diet.

In this work, we present an ultra-high performance liquid chromatography (UHPLC) method for the determination of cyanocobalamin in mealworm larvae. It features the conversion of possible B12 vitamers into cyanocobalamin, enrichment of the target analyte by immune-affinity chromatography and UHPLC separation, which together enable sensitive and high-throughput analysis. UHPLC separation was achieved within 4.0 minutes, by a total runtime of 8 min per analysis. The method was validated in accordance with international guidelines, regarding specificity, accuracy, precision and detection limits, and was found suitable for the intended use. Values for vitamin B12 in mealworm larvae were found to be 3.9 ± 0.4 µg g^{-1}, and thus roughly equivalent to beef. Hence, app. 75 g of mealworm is enough to match the daily required intake of vitamin B12, clearly indicating the usefulness of mealworms to combat vitamin B12 malnutrition.

Insects as protein source for food and feed – consumer perception in Croatia and other European countries

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Demand for food, especially protein component, is constantly increasing and one of possible protein source from sustainable sources are factory farming insects. Beside enough protein production, the Food and Agriculture Organization of the United Nations considers that the cultivation of insects leads to reduced emission of greenhouse gases and ammonia, more efficient protein conversion, lower investments and less demanding technology. European Food Safety Authority provided in October 2015, scientific opinion: Risk profile related to production and consumption of insects as food and feed which includes biological and chemical hazards, allergens and environmental risks. Still, the open question is consumer perception due to new way of animal feeding in terms of acceptability, risk and common knowledge on feed as such.

Croatian Food Agency together with UK, Czech Republic, Spain and Italy was involved in consumer perception survey on using feed with insect proteins added, as a part of PROteINSECT project. This project goal was to examine the feasibility of using insect protein for feed, and it was co-financed by the European Commission. Survey included questions regarding knowledge of the feed content, acceptability of domestic animals feeding with different sources of protein, risk to human health and socio-demographic data.

This paper presents the comparison between consumer perception in Croatia and other four European countries. The results show that there was no difference in the knowledge of animal feed among the respondents from Croatia and other European countries. However, significant differences have occurred in acceptability perception of feeding domestic animals with insect protein and in perception of risk to human health.

Structural and functional characterization of the larval midgut of *Hermetia illucens* for the best exploitation of its bioconversion ability

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The use of insects as a primary agent for organic waste reduction and bioconversion is a promising and sustainable strategy to produce protein that can be used for feed production. The non-pest black soldier fly (BSF), *Hermetia illucens*, is among the most promising insect species to this purpose because of the ability of the larvae to grow on a wide variety of organic substrates and their efficiency in the bioconversion process. Moreover, the high nutritional value of the larvae and pupae makes them an interesting alternative protein source for the production of fish feed.

Although the literature provides information on the rearing methods for BSF and indications on its use for waste treatment, 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.

In the present study we performed a structural and functional characterization of the midgut of last instar larvae grown on two different food substrates, i.e., a standard diet for Diptera and a vegetable mix, 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. They are characterized by different cell types that accomplish digestion and absorption activities (columnar cells), acidification of the midgut lumen (cuprophilic cells), and growth of the epithelium (stem cells). The proteolytic activity is high in the posterior region, where the pH is around 8, and the major activities are due to trypsin- and chymotrypsin-like enzymes. Moreover, the analysis of the amylolytic activity showed that amylases are secreted in the first part of the midgut lumen.

Larvae reared on the vegetable mix do not show alterations in the general morphology of the midgut, but a significant increment in digestive enzyme activities can be observed, especially for those involved in protein digestion.

This work represents the first attempt to characterize from a morphofunctional point of view the larval midgut of *H. illucens* and evaluate the effects of different diets on the

features of this organ. Moreover, it 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)

Consumption of edible insects in Northeast Thailand: Insights into entomophagy in Khon Kaen province

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Background: Whereas Western cultures are believed to currently struggle having edible insects accepted as a source for human food, Thailand is an example par excellence of a nation where insects have been part of the normal diet. This applies especially for the Northeast of Thailand, where Isaan people are known for their high acceptance of entomophagy. Even though the standard of living is rising in Thailand and the country is becoming more westernised, the practice of eating edible insects has become more widespread over the last few years. However, little is known about the actual application of insects within the individual diet.

This survey focuses on entomophagy in the rural Khon Kaen province and urban Khon Kaen in the Northeast of Thailand. It gives insights into general knowledge about entomophagy and personal practice of eating edible insects. The survey concentrated on the kinds of consumption as well as the occasion of consumption. Additionally, subjects were asked to estimate why entomophagy is hardly practiced in the Western World.

Methods: Before starting the survey the used questionnaire was translated from English to Thai with the help of local scientists. In the exact same manner, completed questionnaires were translated from Thai to English and analyzed.

The survey was conducted in and around the province of Khon Kaen in the Northeast of Thailand including the city of Khon Kaen itself. In the rural areas 122 respondents were interviewed by nurses of local Primary Health Care Centers. As people could hardly read or write nurses supported filling in the questionnaires. In comparison, at the Khon Kaen University questionnaires were handed out for example in the canteen and student´s bar. Here 176 respondents filled in the questionnaires without any help.

Results: The average age of the total 298 subjects was 37.7 ± 20.6 years covering a range between 18 and 88 years. The majority of interviewed people was female (65.8%). Distribution of respondents from rural and urban regions was almost equal, amounting to 40.9% of them living in villages of the Khon Kaen province.

In total 94.7% of interviewed people were familiar with the term “entomophagy”. Despite the fact that 83.9% of respondents have had eaten insects before, only 60.7% were aware of their nutritional as well as ecological potential.

There was a significant difference in the preference for certain insects between rural and urban areas. In descending order crickets, giant water bugs, silkworms and grass-

hoppers were the first choice in rural areas, whereas silkworms, crickets, grasshoppers, ants and ant eggs, respectively were predominantly eaten in urban regions.

The majority of interviewed persons affirmed eating whole insects as snacks, independently of living in rural (90.2% agreement) or urban area (71.0% agreement). When being asked whether whole or prepared (ground or crushed) insects are used, significantly more people in rural than in urban areas confirmed doing so (82.0% vs. 26.1%, $p<0.001$ and 27.0% vs.

9.7%, $p<0.001$ respectively), indicating that insects in rural areas are used more often for preparation of meals. This was supported by the fact that significantly more people in rural areas ate insects at home than people in urban areas preferring them as street food. Hardly anyone used isolated insect ingredients such as isolated proteins, fats or minerals.

Independently of living in rural or urban areas, almost half of the subjects did not give any reasons why entomophagy is not practiced in Western Countries. However, 18.2% assumed that the different cultural background and traditions are the main reasons. Climatic reasons were considered to be crucial for 9.7% of asked subjects. 4.5% think that there are none or not enough edible insects in Europe. Interestingly only 1.1% considered taste of importance and 0.6% believed that anxiety is a critical reason for denying entomophagy in the Western World.

Conclusion: This survey underlines the high acceptance of eating insects in the Northeast of Thailand. More than 80% of interviewed subjects have consumed insects. Often they are eaten as snacks. However, in rural areas insects are more often used for preparing dishes. Different cultural and traditional background as well as climatic conditions were believed to mainly hamper the acceptance of eating insects in Western countries.

Changes in antioxidative enzyme activity and malondialdehyde content in zearalenone-treated *Tenebrio molitor* larvae

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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 highest concentrations of ZEN were reported for wheat bran, corn and products thereof (e.g. corn flour, cornflakes). ZEN is genotoxic and responsible of a potent reproductive toxicity in humans and animals. ZEN has been shown to be immunotoxic, hepatonephrotoxic and apoptotic. It has been demonstrated that ZEN and it metabolites induces lipid oxidation and increases the production of malondialdehyde in several cell lines. The fact that *T. molitor* can consume mycotoxin-contaminated feed without any visible adverse effects on larval growth and mortality suggests mycotoxin contamination may go unnoticed by the insect producer. The aim of this study was to determine the association between ZEN with oxidative stress in ZEN-treated *Tenebrio molitor* larvae. In order to evaluate the role of oxidative stress on the effects induced by three weeks administration of ZEN, we measured several enzymatic and non-enzymatic indicators of oxidative stress in *Tenebrio molitor* larvae tissues. The concentration of ZEN in experimental diet was 400 µ g/kg. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) and expressed in terms of malondialdehyde (MDA) content. Enzymatic indicators of oxidative stress were catalase (CAT), and peroxidase (POD) activity. Changes in CAT and POD activity and MDA level in tissues of *Tenebrio molitor* larvae exposed to ZEN was investigated using spectrophotometrically method. The resulting CAT, POD and MDM were compared with protein concentrations in the extract, determined by the modified Bradford method and calculated in mg/protein. Statistical analyses revealed that levels of non-enzymatic marker for oxidative stress, such as MDA was altered by ZEN administration (17.00 ± 2.79 µ M/mg protein; $P < 0.001$) in compared to control group (12.35±0.57 µ M/mg protein). Activities of CAT (0.41±0.01; 0.35±0.01 µ M/mg protein control and experimental larvae respectively; $P< 0.0001$) and POD (0.575±0.034; 0.19±0.01 j.u./mg protein control and experimental larvae respectively; $P< 0.0001$) were altered to by ZEN treatment. In order to better visualize oxidative stress in ZEN-treated *Tenebrio molitor* larvae, the POD activity was compared to the content of MDA and the ratio was determined. The obtained val-

ues indicate the potential antioxidant activity expressed by POD/MDA ratio and the oxidative stress level expressed by the MDA/POD activity. The highest antioxidant activity correlated with the lowest oxidation level was in control group. The antioxidant activity was lower in ZEN-treated larvae, thus was giving higher values of oxidative stress (MDA/POD ratio). In conclusion, diet contaminated with 400 µ g/kg ZEN induces oxidative stress in *T. molitor* larvae, by induces changes in antioxidative enzyme activity and malondialdehyde content.

Zearalenone and bisphenol A induces oxidative stress in *Tenebrio molitor* larvae

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Zearalenone (ZEN) (previously known as F-2 toxin) is a nonsteroidal estrogenic mycotoxin biosynthesized through a polyketide pathway by a variety of Fusarium fungi which are common soil fungi, in temperate and warm countries, and are regular contaminants of cereal crops worldwide. ZEN is commonly found in maize but can be found also in other crops such as wheat, barley, sorghum and rye. ZEN can cause reproductive effects including infertility, abortion, and other breeding problems in livestock. ZEN also causes hepatonephrotoxic, hematoxic, immunotoxic, apoptotic and genotoxic effects in domestic and laboratory animals. It has been demonstrated that ZEN and it metabolites induces lipid oxidation and increases the production of malondialdehyde in several cell lines. The Bisphenol A (BPA) is used as a monomer in the manufacture of polycarbonates and epoxy resins and as an additive in plastics. Polycarbonates are used in food contact materials such as reusable beverage bottles, infant feeding bottles, tableware (plates and mugs) and storage containers. BPA is an endocrine disrupting compound widely spread in our living environment. Numerous studies have demonstrated its endocrine disrupting properties and attributed exposure with cytotoxic, genotoxic, and carcinogenic effects. It was reported that BPA increases the generation of reactive oxygen species (ROS) and induced hepatic damage and mitochondrial dysfunction. BPA induced a significant increase in oxidative stress, which is accompanied by marked alterations in total antioxidant capacity (TAC). The aim of this study was to determine the association between ZEN and BPA exposure on oxidative stress in ZEN-BPA treated *Tenebrio molitor* larvae. In order to evaluate the role of oxidative stress on the effects induced by three weeks administration of ZEN and BPA and we measured several enzymatic and non-enzymatic indicators of oxidative stress in *Tenebrio molitor* larvae tissues. The concentration of ZEN and BPA in experimental diet (barley) was 400 and 600 µg/kg respectively. The lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) and expressed in terms of malondialdehyde (MDA) content. Enzymatic indicators of oxidative stress were catalase (CAT) and peroxidase (POD) activity. Changes in CAT and POD activity and MDA level in tissues of *Tenebrio molitor* larvae exposed to ZEN and BPA were investigated using spectrophotometrically method. The resulting CAT, POD and MDM were compared with protein concentrations in the extract and determined by the modified Bradford method and calculated in mg per

protein. Statistical analyses revealed that levels of non-enzymatic marker for oxidative stress, such as MDA was altered by ZEN and BPA administration (14.34 ± 2.19 µ M/mg protein; P<0.001) in compared to control group (12.35±0.57 µ M/mg protein). Activities of CAT (0.41 ±0.01; 0.37±0.004 µ M/mg protein control and experimental larvae respectively; P< 0.001) and POD (0.57±0.03; 0.23±0.016 j.u./mg protein control and experimental larvae respectively; P<0.0001) were altered to by ZEN and BPA treatment. In order to better visualize oxidative stress in ZEN-BPA-treated *Tenebrio molitor* larvae, the POD activity was compared to the content of MDA and the ratio was determined. The obtained values indicate the potential antioxidant activity expressed by POD/MDA ratio and the oxidative stress level expressed by the MDA/POD activity. The highest antioxidant activity correlated with the lowest oxidation level was in control group. The antioxidant activity was lower in ZEN-BPA-treated larvae, thus was giving higher values of oxidative stress (MDA/POD ratio). In conclusion, diet contaminated with 400 µ g/kg ZEN and 600 µ g/kg BPA induces oxidative stress in *T. molitor* larvae, by induces changes in antioxidative enzyme activity and malondialdehyde content

Food based on edible insects is a challenge for the official food control authorities

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What still may sound futuristic at the first moment, might soon become an alternative to "traditional" food: more and more often we encounter food insects for human consumption, mainly offered on the Internet. In Asia, Africa, Latin America, or Australia insects have long been one of the main foodstuffs. In the European Union scepticism against the "unaccustomed", rejection or even disgust may be possible reasons why the marketing of edible insects is still a niche. Nevertheless, insect-based foods are expected to grow rapidly in Europe. Media exposure and public interest have recently increased immensely, probably also due to the marketing strategies of the participating stakeholders. In addition, insects are discussed as an alternative source of protein to ensure the nutrition of a growing world population and to have a potential positive ecological and economic balance compared to conventional animal breeding. The amended Regulation on Novel Foods, which will be valid from January 2018, provides for a centralized, simplified as well as accelerated authorization procedure, and is therefore expected to lead to an increasing offer of corresponding products on the German market. Thus, it becomes obvious that official monitoring of food insects is also a new challenge. The CVUA (Chemistry and Veterinary Research Office) Freiburg has therefore been working on this topic for some time.

Decontamination of *Tenebrio molitor* flour by dry heat: Impact on protein functionality, solubility, composition and structure

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Recently, insects have gained more and more attention in Europe as an underexploited sustainable animal protein for food and feed. But western consumers may be reluctant to accept insects as a legitimate protein. Extracting insect proteins for human food products could be a useful way of increasing acceptability among wary consumers. However, supplementing food products with insects requires extensive knowledge on the proteins properties. Further, effects on protein properties induced by required sanitation steps during processing need to be investigated.

Aim of this study was to investigate the applicability of thermal processing of insect flour from *Tenebrio molitor* for the reliable reduction of the total microbial load within a short-term treatment with varying temperatures up to 140°C. Besides investigating the decontamination efficacy, process-induced impact on product color, techno-functionality, protein solubility, composition and structure was analysed.

The total microbial load of the *Tenebrio* flour of $6.3 \cdot 10^6$ cfu/g was reduced to $3.5 \cdot 10^6$ (40°C), $2.8 \cdot 10^5$ (60°C), $2.1 \cdot 10^5$ (80°C) and $2.3 \cdot 10^4$ (100°C) cfu/g whereas dry heat with temperatures of 120 and 140°C resulted in a complete microbial inactivation. When compared to the untreated sample solubility of the contained proteins at pH 4 linearly increased to 106% upon heat treatment at up to 80°C and was reduced to 44% by further increasing the temperature to up to 140°C. Decrease in protein solubility with elevated temperature was less pronounced at pH 10, where proteins were most soluble. Water binding capacity linearly decreased from 0.79 to 0.55 g_{water}/g whereas oil binding capacity was not affected. Gel electrophoresis revealed a temperature-dependent decrease of all protein fractions at pH 4 and 10 with temperatures above 100°C. Tryptophan fluorescence spectra of the protein extracts supplied first evidence of heat-induced structural protein modification whereas gel electrophoresis indicated changes in the protein composition.

Evaluation of the mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) and the waxmoth *Galleria mellonella* (Lepidoptera: Pyralidae) larvae as new potential sources of ACE inhibitory peptides

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In response to the growing food demand of the global population that continues to rise and has been estimated 9.6 billion people in 2050, edible insects have been proposed by Food and Agriculture Organization of the United Nations (FAO) as a promising food and feed alternative to meat and fish. Generally, edible insects provide sufficient energy and proteins of high quality. The content of essential amino acids (e.g. Met, Lys, Leu) meets requirements for human diet in most edible insects, suggesting their potential as a source of bioactive peptides able to exert several physiological activities in human body. In the last decades, peptides with a wide activity spectrum, such as antitumor, anti-obesity, antioxidant and especially anti-microbial activity, have been isolated from insects. Among these bioactive compounds angiotensin converting enzyme (ACE) inhibitory peptides are of great interest for heart disease prevention, since they are able to reduce blood pressure by inhibiting ACE that plays a key role in systemic renin-angiotensin system. ACE inhibitory peptides can be obtained by hydrolysis of the protein extracts mediated by different proteolytic enzymes, such as the gastrointestinal proteases (trypsin, α-chymotrypsin and pepsin) which can be used alone or combined together to simulate digestion process that occurs in human gastrointestinal tract. ACE inhibitory activity has been investigated in gastrointestinal protein hydrolysates from the yellow mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae) and the waxmoth *Galleria mellonella* (Lepidoptera: Pyralidae) recently listed by EFSA among the most potential edible insect species to be reared on an industrial scale for food and feed in European Union. A higher *in vitro* ACE inhibitory activity has been detected in gastrointestinal protein hydrolysates than in crude protein extracts from *T. molitor* (IC_{50} = 0.097 mg/mL *vs* 0.720 mg/mL in crude extract) and *G. mellonella* (IC_{50} = 0.046 mg/mL *vs* 5.413 mg/mL in crude extract). These preliminary data confirmed the potential of these two edible insect species as source of bioactive peptides with high antihypertensive capacity, suggesting their future application in food industry as fortified food or as health supplement for human diet.

Fat content and fatty acid composition of the yellow meal worm *Tenebrio Molitor* (Coleoptera: Tenebrionidae) reared on different diets

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Tenebrio molitor (Coleoptera: Tenebrionidae) represents an interesting resource of polyunsatured fatty acids (PUFA), especially n-6 and n-3 fatty acids, involved in the prevention of many pathological conditions, mainly cardiovascular diseases. The possibility to modify fat content and fatty acid composition of the yellow mealworms (larvae and pupae) through different feeding substrates have been investigated in the future prospective to apply this coleopteran as diet supplement for human health. Despite the different fat content in the six different breeding diets used, *Tenebrio molitor* larvae and pupae contained a constant total fat percentage (> 34% in larvae and > 30% in pupae); no significative difference of fat content was found between male and female pupae. Diversely fatty acid composition differed both in larvae and pupae according to the feeding substrates. *T. molitor* fed on the diets based on 100% bread and 100% oat flour showed SFA, PUFA percentages, and an n-6/n-3 ratio more suitable for human consumption but does not guarantee advantageous larval development times for mealworm mass-rearing production.

The most prevalent FA detected in *T. molitor* larvae and pupae are: myristic, palmitic and stearic acids among the SFA, oleic (the most abundant > 40%) and palmitoleic acids among the MUFA, α-linoleic and α-linolenic acids among the PUFA. Generally, *T. molitor* larvae reared on classical substrates based on cereals (oat, corn and wheat flours) or chickpea flour showed a lower n-6/n-3 ratio (31.54-40.53) than pupae (32.09-44.06). Among these diets, those one based on beer yeast, wheat flour and oat flour assured in the same time the best balance between fat composition (comparable to pork or bovine) and favourable insect growth conditions.

The possibility to enhance polyunsaturated acids (PUFA) to reduce the n-6/n-3 ratio, was evaluated in *T. molitor* mealworms larvae (the most promising developmental stage for a mass-rearing) by adding linseed, as source of n-3 fatty acids. Larvae from all enriched diets evidenced a significant decrease of saturated fatty acids (SFA) and an increase of PUFA contents, especially n-3 acid, with a decrease of n-6/n-3 ratio to optimal values (0.76-1.83) suitable for the prevention of cardiovascular diseases.

Epigenetic inhibitors are affecting life traits of the pea aphid

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The pea aphid, *Acyrthosiphon pisum* (Harris), is an agricultural pest and laboratory model for research studies. It has been shown in different organisms that disruption of epigenetic regulation with inhibitory compounds can lead to an alteration in lifespan, development and fecundity.

In aphids, little is known of the effect of impaired DNA methylation and until today the role of alteration of histone acetylation has not been investigated.

The aim of this study is to clarify whether changes in epigenetic processes (e.g. DNA methylation, histone acetylation) have an impact on aphids' life traits.

Inhibitors of histone deacetylases (suberoylanilide hydroxamic acid, SAHA), histone acetyltransferases (epigenetic multiple ligand) and DNA-methyltransferases (5-Azacytidin) were used for feeding assays.

Furthermore, gene regulation involved in epigenetic mechanisms is tested in order to examine effect of epigenetic compounds. All the tested compounds used in this study reduced survival and delayed start of reproduction of the pea aphids. In addition, inhibition of histone deacetylation and DNA methylation decreased number of offspring. This study elucidates epigenetic regulation in the pea aphid which could lead to discovery of new targets for pest control.

Processing the fat of the black soldier fly *Hermetia illucens*

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The larvae of the black soldier fly (*Hermetia illucens*) were dried and mechanically extracted by oil seed press to remove the fat. As a product the protein enriched material was gained. The fat was fully refined. Crude and refined fat were characterized. Practical Application: The fat-removed and protein enriched material from processing the larvae of the black soldier fly is a good source to feed farm animals and pets.

The removed fat can be used as well for feeding farm animals, as lubricant or to produce soaps and detergents.

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