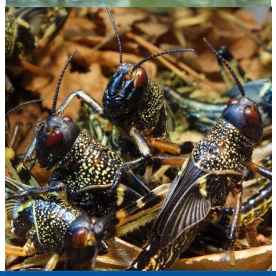
Heft 105 (2021)



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Bornimer Agrartechnische Berichte

INSECTA 2021

International Conference

Book of Abstracts

08th – 09th September 2021
Magdeburg, Germany

ATB

INSECTA 2021

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Heft 105
Potsdam 2021

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Published by:

Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB)
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August 2021

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Dr. Shikha Ojha
Dr.-Ing. habil. Oliver K. Schlüter

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(from top to bottom)

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

Layout:

Beate Spehr

Published by the Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB) with the support of the Federal Ministry of Food and Agriculture (BMEL) as well as of the Ministry of Science, Research and Culture of the State Brandenburg (MWFK).

ISSN 0947-7314

Preface

On September 8 and 9, 2021 the INSECTA conference is organized in Magdeburg by Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM) and Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB). In 2015, PPM initiated the first national INSECTA in Magdeburg and since 2016, the INSECTA has been jointly organized by PPM and ATB. A major goal of the conference is to bring national and international experts from industry and academia together to exchange and share their experiences and research results. We aim to continue the success of recent congresses and to ensure that INSECTA will foster interdisciplinary collaboration in insect science and technology.

For the first time, INSECTA conference will offer the possibility of online participation. The organizers hope to be able to hold INSECTA 2021 conference as a partially on-site event and would like to offer all interested participants who are unable to travel the opportunity to be present online. We have the opportunity of a more intensive and constructive exchange of ideas and the opportunity to establish contacts with new cooperation partners. As an added value, all participants will be given the possibility to attend recorded parallel sessions or individual presentations after the event. Poster presentations will be available on-site and in online gallery. It is always possible to contact the authors for questions and further discussion. There will also be noticeable changes for the participants on-site. Most of them are already known from pandemic prevention as social distancing, testing and tracking list.

We are very much looking forward to welcoming our visitors to Johanniskirche, a place that is historically very connected to Magdeburg: the Johanniskirche is the oldest merchant's church in Germany, Martin Luther spoke to the residents here and it was also home- and grave- church of Otto von Guericke, mayor and scientist in Magdeburg. Unfortunately, we can only have limited participants with us on-site. The get-togethers and evening events were also always important for the networking and we would like to offer both in a small framework.

Since the options of exchange were limited last year, we are looking forward to many new ideas and lively discussions on insects as a novel source of bio-materials and new strategies in unraveling its potential for food, feed and other specialized applications, as well as meeting friends and cooperation partners.

We would like to thank all the partners and supporters at the ATB and PPM and our sponsors for their immense support in the new hybrid way of INSECTA conference this year.

We would also like to thank the speakers and participants who have helped to make INSECTA an important platform for discussions of new ideas and exchange of knowledge!

Sara Hadjiali, Oliver Schlüter, Thomas Piofczyk Magdeburg in August 2021

Organizing Team

Dr.-Ing. Sara Hadjiali
Dr.-Ing. Thomas Piofczyk
M. Sc. Sindy Goldhahn
Anke Rehwald-Faupel
Pia Hadouch
Dr.-Ing. Julia Durek
Dr. Shikha Ojha
Dr.-Ing. habil. Oliver K. Schlüter

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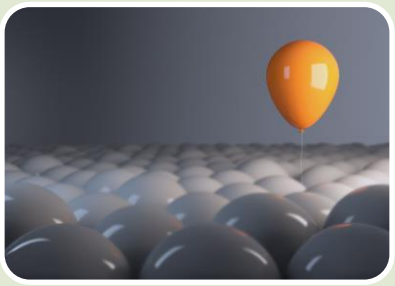

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Berliner Chaussee 66 | 39114 Magdeburg
Dr.-Ing. Sara Hadjiali | E-Mail: hadjiali@ppm-magdeburg.de |Tel.: +49-391-8189-132

ATB

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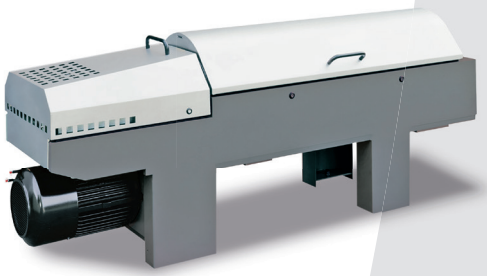

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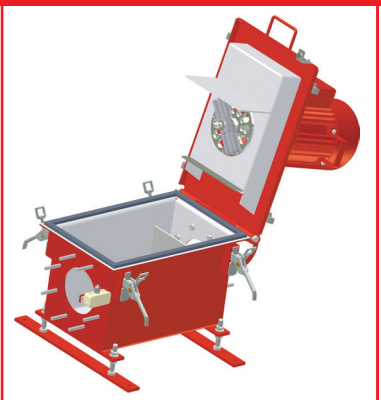

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Heise Ölmühlen

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The VDI is an association for technically and scientifically active people (VDI: Association of German Engineers) and is one of the largest technically oriented clubs and associations in the world.

The VDI Magdeburger Bezirksverein supports the INSECTA conference since 2015 and grants the “VDI Best Young Scientists Presentation Award” every year. It is divided into 6 district groups and many thematically different working groups and has offers for all ages. One group related to INSECTA conference is the Arbeitskreis Nachwachsende Rohstoffe (circle of experts for renewable resources) that is managed by Sara Hadjiali. VDI Magdeburger Bezirksverein is organised by voluntary works.

The VDI Magdeburger Bezirksverein is supported in its work by numerous companies in the region and forms an important network and the VDI is also international networked.

Also VDI Magdeburger Bezirksverein has a sponsorship award for students at the universities in Magdeburg every year and the Gruson plaque of honor for members who deserved in the technical development and the work of VDI Magdeburger Bezirksverein Magdeburg.

More information can be found at homepage:

https://www.vdi.de/ueber-uns/vor-ort/bezirksvereine/magdeburger-bezirksverein

Historical overview of the Johanniskirche in Magdeburg

The former parish and council church of Sankt Johannis goes down in the history of German churches as the first "eccelsia mercatorum" so-called merchant church of local merchants in Germany (10th / 11th century). In 1188 she fell victim to the great city fire. With the construction of the two west towers from 1207 to 1238, the double tower front typical of Magdeburg's parish churches was created for the first time. Around 1300 it was converted into a three-aisled Gothic hall church. In 1451 the church burned down as a result of a lightning strike in the tower front.

In 1524 Martin Luther gave his sermon here “On true and false justice”, as a result of which the council and the city profess Protestantism.

With the destruction of the city in the Thirty Years War (1631), the Johanniskirche also fell to rubble. The successor building can only be inaugurated again on the 1st of Advent 1670. The church's furnishings included the baroque pulpit by the Magdeburg sculptor Tobias Wilhelmi from 1669 and the large organ by the Hamburg master organ builder Arp Schnitger from 1695.

From 1806 to 1807 Napoleonic troops occupy Prussia's strongest fortress, Magdeburg. The church becomes a horse stable. From 1832 to 1851 the Ratskirche is also telegraph station no. 14 on the Royal Prussian Optical Telegraph Line Berlin–Coeln–Coblenz.

During the bombing of Magdeburg on September 28, 1944 and January 16, 1945, the Johanniskirche fell into ruins again in its history. On October 2, 1999, the church was restored as a festival and event hall. The church roof has been restored in its old dimensions.

Otto von Guericke, Mayor of Magdeburg, scientist and inventor, was buried in the crypt on the north side of the Johanniskirche.

The Martin Luther monument in front of the church is the work of the sculptor Emil Hundrieser (1886). The bronze door with the two accompanying solitary sculptures "War" and "Peace" were made by the Magdeburg sculptor Heinrich Apel and want to commemorate the destruction of Magdeburg in 1631 and 1945.

Source and more information: https://www.mvgm.de/de/johanniskirche/

Program

Tuesday September 7

07:00 p.m	Get Together	Ratskeller-Biergarten	

Wednesday September 8

08:00 a.m	Registration	Johanniskirche	
09:00 a.m.	Sara Hadjiali	Welcome in Magdeburg	Pilot Pflanzenöltechnologie Magdeburg e.V.
	Tino Sorge	Greetings Words	Member of Bundestag
	Nils Th. Grabowski, et al. keynote	Veterinary duties in productive insects	Institute for Food Quality and Food Safety, Hannover University of Veterinary Medicine, Hannover, Germany
	Nanna Roos, et al. keynote	Insect farming for better nutrition, health and livelihoods in Africa	Department of Nutrition Exercise and Sports (NEXS), University of Copenhagen, Denmark
	Jesus González	Company Presentation: Phileo by Lesaffre	SI Lesaffre, Phileo division
10:30 a.m.	**Coffee Break, Poster Exibition, Sponsors Exhibition**		
11:00 a.m.	Itai Opatovsky, et al.	The super power of fungi: unraveling their metabolic effects on black soldier fly larvae	Laboratory of Insect Nutrition and Metabolism, The Department of Nutrition and Natural Products, MIGAL – Galilee Research Centre, Kiryat Shmona, Israel
	Jesus González, et al.	Zootechnical performance improvement by Live Yeast Probiotics on Black Soldier Fly Fed (*Hermetia illucens*)	Phileo by Lesaffre, Marcq-en-Baroeul, France
	Thomas Klammsteiner, et al.	Individual density and rearing scale influence temperature profiles during black soldier fly rearing	Dept. of Microbiology, Univ. of Innsbruck, Innsbruck, AT
	Bernd Pütz, Artur Kühl	Dry processing of insects – how does it work in theory and Praxis	Maschinenfabrik Reinartz GmbH & Co. KG, Neuss, Germany
	Luc Sweers, et al.	The potential of dry fractionation for insect processing	Food Quality and Design Group, Wageningen University & Research, Wageningen, The Netherlands
	Maryia Mishyna, et al.	Fractionation of lesser mealworm (*Alphitobius diaperinus*) and characteristics of a cream layer	Food Quality and Design group, Wageningen University, Wageningen, the Netherlands
	Laurence Auger, et al.	Host microbiota profile, interactions, and vertical transmission across two generations of *Hermetia illucens*	Université Laval, Department of Biology, Institute for Integrative and Systems Biology (IBIS), Québec, Canada
	Ellen Gorrens, et al.	Variation in the bacterial community composition of black soldier fly larvae (*Hermetia illucens*) from consecutive, industrial cycles	KU Leuven, Department of Microbial and Molecular Systems, Research Group for Insect Production and Processing, Geel, Belgium

	Laurens Broeckx, et al.	Influence of organic side stream nutritional composition on the performance of *Hermetia illucens* larvae	Thomas More University of Applied Sciences, RADIUS, Geel, Belgium
	Piotr Bulak, et al.	The use of insect for cleaning the environment: ento-moremediation	Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland
	Patrick Sudwischer	Development of a method to determine chitin in insect protein meal and insect farming by-products	Development of a method to determine chitin in insect protein meal and insect farming by-products
	Thomas Piofczyk. et al.	Extraction of chitosan from insect chitin	Pilot Pflanzenöltechnologie Magdeburg e.V. (PPM), Magdeburg, Germany
13:05 p.m.	**Lunch Break, Poster Exibition, Sponsors Exhibition**		
14:05 p.m.	Mark Benecke	Insects, sustainability and more	Öffentlich bestellter und vereidigter Sachverständiger für kriminaltechnische Sicherung, Untersuchung und Auswertung von biologischen Spuren (IHK Köln), Germany International Forensic Research & Consulting
15:00 p.m.	Nina Kröncke, Rainer Benning	Determination of moisture, protein and fat content in living mealworm larvae (*Tenebrio molitor*) by near infrared reflectance spectroscopy (NIRS)	Institute of Food Technology and Bioprocess Engineering, University of Applied Sciences Bremerhaven, Bremerhaven, Germany
	John Ames	Would An Oat Milk Producing Farm See A (Financial) Bonus To Raising Black Soldier Fly On Its Residues?	madebymade Gmbh, Pegau, Germany
	Franco Honegger, Andreas Baumann	The potential of data in an industrial insect plant	Bühler AG, Uzwil, Switzerland
	Nathan Meijer, et al.	Effects of pesticide residues on insects reared for food and feed	Wageningen Food Safety Research (WFSR), Wageningen, The Netherlands
	Cédric Auriol	The First Novel Food Authorisation for Insect: a historical and necessary milestone for the whole industry	SAS EAP Group – Agronutris, Saint-Orens de Gameville, France
	Leen Van Campenhout	Inoculation experiments with food pathogens during insect rearing and during heat treatment of frass	KU Leuven, Department of Microbial and Molecular Systems (M2S), Research Group for Insect Production and Processing, Geel, Belgium
16:00 p.m.	**Coffee Break, Poster Exibition, Sponsors Exhibition**		
16:30 p.m.	Kriti Shrestha, et al.	Results of Long-Term Selective Breeding of *Hermetia illucens* for Industrial Applications	Protix B.V., Dongen, The Netherlands
	Sara Bellezza Oddon, et al.	Isoenergetic, isonitrogenous, and semi-purified diets for lipid requirement determination in *Hermetia illuc*ens larvae	Department of Agricultural, Forest and Food Sciences, University of Turin, Italy
	Antti Vasala, Ari Riihimaa	Bioreactor-based mass cultivation of insect larvae	Entoprot Ltd, Oulu, Finland

	Giulia Leni, et al.	Agro-food leftovers as insect feedstock for producing carotenoid-rich *Hermetia illucens*	Department of Food and Drug, University of Parma, Parma, Italy
	Lennard Pisa, et al.	Bioconversion of chicken manure by housefly larvae (*Musca domestica L.*); larval performance and substrate conversion in relation to sterilization and carbohydrate addition	Animal Nutrition Group, Wageningen University & Research, The Netherlands
	Lotte Frooninckx, et al.	Use of grid to improve egg laying efficiency of *Tenebrio molitor* beetles	Thomas More University of Applied Sciences, RADIUS, Geel, Belgium
17:30 p.m.	**End of Lectures**		
19:30 p.m.	**Evening**	**Ratskeller-Biergarten**	

Thursday September 9

08:00 a.m Johanniskirche

09:00 a.m.	Alessandro Monaco Keynote	Looking for Insects in the Regulatory Forest: Critical Aspects and Challenges Posed by the Regulatory Environment applicable to Insects in the European Union	Universität Bayreuth, Fakultät 7, Campus Kulmbach, Germany
	Dennis Oonincx Keynote	Sustainability in the insect sector	Animal Nutrition Group, Wageningen University & Research, The Netherlands
	Stefan Kirchner	Company Presentation GEA	GEA
10:15 a.m.	Nina Parry, Chris Weldon	The effect of chicken manure and pre-consumer waste on black soldier fly (*Hermetia illucens*) larval performance at industrial scale	University of Pretoria, Pretoria, South Africa
	Anna Valentina Luparelli, et al.	Modification in composition of black soldier fly puparium, prepupae and adults after *Lactobacillus* fermentation	Department of Food Science and Drug, University of Parma, Parma, Italy
	Shahida Anusha Siddiqui, et al.	Automated, modular systems for rearing *Hermetia illucens* larvae – design considerations and sustainability	Technical University of Munich Campus Straubing for Biotechnology and Sustainability, Straubing, Germany
	Lenka Kouřimská, et al.	Purines in edible insects and their suitability for people suffering gout	Department of Microbiology, Nutrition and Dietetics, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague, Czech Republic
	Catriona Lakemond, et al.	Benchmarking insects suitability in food supply chains under crisis conditions	Food Quality & Design, Wageningen University, Wageningen, The Netherlands
	Verena Böschen, Patrick Sudwischer	Boundary conditions in insect engineering (from air humidification to the fractionation to the end product)	Forschungsinstitut Futtermitteltechnik der IFF, Braunschweig, Germany

11:15 a.m. Coffee Break, Poster Exibition, Sponsors Exhibition

11:40 a.m.	E.F. Hoek – van den Hil, et al.	Safety of black soldier fly (*Hermetia illucens*) larvae reared on different biowaste substrates	Wageningen Food Safety Research (WFSR), Wageningen University & Research, Wageningen, The Netherlands
	Ren Sakurai, Akihiro Iijima	Target marketing on edible insects' business	Graduate School of Regional Policy, Takasaki City University of Economics, Gunma, Japan
	Cecilia Lalander, et al.	Feasibility of small-scale BSFL composting in the EU	Department of Energy and Technology, Swedish University of Agricultural Sciences, Uppsala, Sweden
	Mik Van Der Borght, et al.	Why Black Soldier Fly Larvae Protein Determinations Bug(ged) Researchers	KU Leuven, Department of Microbial and Molecular Systems (M2S), Research Group for Insect Production and Processing, Geel, Belgium
	Aman Paul, et al.	Anti-arthritic activity of black soldier fly (*Hermetia illucens*) larvae protein derivatives	Centre of Oxygen, Research and Development, University of Liege, Liege, Belgium
	Andreas Baur, et al.	Monitoring of *Tenebrio Molitor* pupae based on Region Based – Convolutional Neural Networks (R-CNN)	Institute of Fluid Mechanics, FAU Erlangen – Nuremberg, Erlangen, Germany

01:30 p.m. Conclusion, Awards, Invitation to 2022

02:00 p.m. End of Conference

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Poster Exhibition

Brigitte R. Paulicks, et al.	Experimental Investigations about the Dietary Protein Requirement of the Yellow Mealworm (*Tenebrio molitor*)	Chair of Animal Nutrition, TUM School of Life Sciences, Freising, Germany
Jeroen De Smet, et al.	The impact of genotype-environment interactions on the microbiota in the larvae of the black soldier fly (*Hermetia illucens*)	KU Leuven, Department of Microbial and Molecular Systems, Research Group for Insect Production and Processing, Geel, Belgium
Sabine Van Miert	ValuSect: Valuable inSects	Thomas More University of Applied Sciences, RADIUS, Belgium
Maricruz Bermúdez-Serrano, et al.	Exploring the production and export potential of cricket powder in Costa Rica	SEPT Competence Center, Leipzig University, Leipzig, Germany
Pascual J.J., et al.	Nutritive value of wheat bran in yellow mealworms (*Tenebrio molitor*): towards feed efficiency optimization	Feedect, Valencia, Spain
Nils Th. Grabowski, et al.	Growth patterns in small-scale farmed edible crickets in Germany	Institute for Food Quality and Food Safety, Hannover University of Veterinary Medicine, Hannover, Germany

Nils Th. Grabowski, et al.	Small-scale food cricket production in Thailand, Cambodia, and Germany using local feed sources	Institute for Food Quality and Food Safety, Hannover University of Veterinary Medicine, Hannover, Germany
Costanza Jucker, et al.	Black soldier fly (BSF) production using the organic fraction of municipal solid waste.	Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy
Michal Kurečka, et al.	Carrot supplement enhanced the levels of lipophilic vitamins in Jamaican field crickets – preliminary results	Department of Zoology and Fisheries, Czech University of Life Sciences Prague, Prague, Czech Republic
Patrick Klüber, et al.	Strategies and suggestions for optimizing *Hermetia illucens* rearing	Fraunhofer Institute for Molecular Biology and Applied Ecology, Giessen, Germany
Petra Škvorová, et al.	Influence of feed on nutritional quality *Gryllus assimilis*	Department of Microbiology, Nutrition and Dietetics, Czech University of Life Sciences, Prague, Czech Republic
Loïc Detilleux, et al.	In what context do you want to eat edible insects?	Economics and Rural Development, Gembloux Agro-Bio Tech – University of Liège, Gembloux, Belgium
Noor Van Looveren, et al.	Impact of heat treatment on the microbiological composition and safety of frass of black soldier fly larvae (*Hermetia illucens*)	KU Leuven, Department of Microbial and Molecular Systems, Research Group for Insect Production and Processing, Geel, Belgium
Manfred Mielenz, et al.	Effects of sewage sludge recyclate supplementation of substrate on cadmium, lead and iron contents in BSF larvae	Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany
Johann Detilleux, et al.	Effect of Black Soldier Fly larvae on horse fecal emission of me-thane	FARAH, Production animale durable, University of Liège, Liège, Belgium
Daniel Gärttling, et al.	Effects of Black Soldier Fly (*Hermetia illucens*) by-product application on the whole-cycle performance of fungus gnats (*Diptera: Sciaridae*)	University of Hohenheim – Department of Applied Entomology, Hohenheim, Germany
Monika Kaczor, et al.	Biovalorization of digested municipal sewage sludge: *Hermetia illucens* vs *Tenebrio molitor*	Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland
Thomas Klammsteiner, et al.	Frass to gas: industrial insect rearing residues as co-substrates for anaerobic digestion	Department of Microbiology, University of Innsbruck, Innsbruck, Austria
Consol Kubayi, et al.	Frass from black soldier fly larvae as a valuable fertilizer and biopesticide for crops	SARChI-Chair On Biodiversity Value and Change, Department of Biology , University of Venda, Thohoyandou, South Africa
Carolin Lappöhn, Michael W. Wolff	An economic purification platform for insect-derived antimicrobial peptides	Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Giessen, Germany
Qi Li, et al.	Termites farming: a new horizon to bioconvert lignin-rich residues into high-value products	Ÿnsect, R&D department, Evry, France
Sayed Mahdi et al.	Modular environmental and economic assessment applied to the production of *Hermetia illucens*	German institute of food technologies – DIL, Quakenbrück, Germany
Freek IJdema, et al.	Substrate fermentation evaluated as a strategy to improve the ω-3 content of black soldier fly larvae (*Hermetia illucens*)	KU Leuven, Department of Microbial and Molecular Systems, Research Group for Insect Production and Processing, Geel, Belgium
Claudia Keil, et al.	Systematic studies on the antioxidant capacity and volatile compound profile of yellow mealworm larvae (*Tenebrio molitor L.*) under different drying regimes	TU Berlin, Institute of Food Technology and Food Chemistry, Department Food Chemistry and Toxicology, Berlin, Germany

Rebeca Ramos-Bueno, Maria Jose González-Fernández	Protein hydrolysates from edible insects: agricultural applications	Tecnova Technology Centre, Almeria, Spain
Patrick Sudwischer, Verena Böschen	The influence of the Maillard reaction on insect products and their nutritional score	Forschungsinstitut Futtermitteltechnik der IFF, Braunschweig, Deutschland
David Terrey, Jack James, et al. presented by Nuria Martin Tome	Palatability improvement potential of Black Soldier Fly Larvae Protein Hydrolysate in Pacific White Shrimp diets	Protix B.V., Dongen, The Netherlands
Dries Vandeweyer, et al.	Survival of black soldier fly larvae (*Hermetia illucens*) in water at different temperatures: potential for storage and transport	KU Leuven, Department of Microbial and Molecular Systems, Research Group for Insect Production and Processing, Geel, Belgium
Harald Wedwitschka, Heinrich Katz	Competitive Insect Products Feedstock suitability assessment for *Hermetia* rearing and waste treatment of insect farming residues by anaerobic digestion	Deutsches Biomasseforschungszentrum gemeinnützige GmbH, Leipzig, Germany
Marios Psarianos, et al.	Alternative processes for the production of chitosan from house crickets (*Acheta domesticus*)	Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
Giacomo Rossi, et al.	Description and characterization of the fluorescence excitation emission matrix of powders from selected edible Orthoptera species: A chemometric approach	Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany
Marwa Shumo, et al.	A molecular survey of bacterial species in the guts of black soldier fly larvae (*Hermetia illucens*) reared on two urban organic waste streams in Kenya	Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany

Veterinary duties in productive insects

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Productive insects are insects farmed for a specific use, e.g. feed, food, industry, waste or pest management. Farming animals means handling large groups of animals, and this poses a series of challenges known also from common livestock.

Veterinarians have been attending pets, zoo and wildlife animals, and aquaculture and ordinary livestock species. They have three focuses: a) animal health, b) animal welfare, and c) sustainability. In the case of livestock species destined for human consumption, vets work on any stage of the farm to fork chain, particularly the primary production and the public health sector. They act as bond between what animals need and what farmers can provide to create high yields from healthy and not welfare-compromised animals. Veterinarians have important duties related to official inspection of foodstuffs, including monitoring of foodborne diseases and other zoonoses.

On one hand, many insect farmers have not sought advice with veterinarians, as they associate them with vertebrates' health care. On the other hand, current veterinary training does not include productive insects, but this is changing, e.g. in Germany.

For productive insects, vets and farmers should work together like for other livestock, i.e. vets must develop herd management strategies for animal health and animal welfare. However, and in contrast to the latter-named, corresponding information is scarce for productive insects under farm conditions. Presently, animal health monitoring is based on herd observation, isolation of affected animal groups, and protection of the farm colonies. Animal welfare mainly refers to observing the five freedoms and a killing method, which produces as less stress as possible (freezing). As knowledge is created, it will have to be integrated into this herd management.

As a response to this need, the International Network for Productive Insects' Health and Welfare (INPIHW) was founded in 2019. Mainly consisting of veterinarians and biologists, it is emphasizing this counselor position by research on one side, and by reach-out and capacity building on the other.

Looking for insects in the regulatory forest: critical aspects and challenges posed by the regulatory environment applicable to insects in the European Union

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Following the approval of the first insect for human consumption (*Tenebrio molitor larva*) in May 2021, preliminary conclusions on the applicability of Regulation (EU) 2015/2283 on novel foods (the “Novel Food Regulation”) to insects and products thereof can finally be drawn.

The presentation aims at offering to food business operators tools to navigate the European legal framework applicable to insects, clarifying grey areas and opening up the discussion on critical aspects and current challenges.

Following a “Farm to Fork” approach, the journey of insects through the regulatory environment of the European Union will be presented focusing on both pre-market approval and post-market requirements.

The difference between authorization and notification procedures under the Novel Food Regulation will be analyzed by clarifying important legal definitions (“Novel Foods”, “Traditional Foods from Third Countries”, “History of Safe Food Use”). In particular, the role of EFSA will be analyzed and available guidelines for the risk assessment of insects and products thereof will be examined, in light of the publicly available information of the *Tenebrio molitor* dossier.

Insights from other applicable legislation (e.g. hygienic requirements for insects breeding, labelling of final products) will also be considered, to provide food business operators with a comprehensive and updated analysis of all legal provisions currently relevant for insects’ production, commercialization and consumption in the European Union.

Sustainability in the insect sector

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One of the main arguments for proposing the use of insects as food, or as feed, is their low environmental impact. Whereas this argument was used lightly at first, in the last decade more and more solid data in the form of Life Cycle Assessments (LCA) has become available. The concept and the limitations of the LCA-methodology are briefly explained, after which the most studied impact categories (Energy use, greenhouse gas emissions and land use) are discussed using mealworms, crickets and flies as examples. These examples are put in perspective by comparing to suitable benchmarks and by clarifying the relevance of societal and logistical context. While in the current insect production systems land use is relatively low, the greenhouse gas emissions, associated with global warming and especially the energy use, associated with fossil fuel depletion, are aspects of concern. A first driver of this impact is the need for extensive climate control. Technological advancements in insect production systems, for in-stance recirculation of heat, can reduce this impact. Moreover, structural changes such as the use of renewable energy sources can further help to limit fossil fuel depletion. A second main driver of environmental impact of insect production is the utilized feed. Several approaches can help mitigate this environmental impact. Firstly, feed sources that are unsuitable for conventional livestock can in certain cases be safely redirected into insect production systems. For instance, mycotoxin contaminated feed which might be safely valorized in insect production systems, thereby retaining these materials within our food systems, if legislation would permit. Similar to conventional live-stock, feed utilization efficiency will improve as we learn more on the nutrient requirements of insects. This will reduce the required input per kg of insect, allow a better utilization of available by-products, and decrease frass production. Lastly, an efficient use of insect-derived products, and by-products, including the frass, will help retain nutrients in production cycles, increase circularity and decrease environmental impact. This approach not only increases environmental sustainability, it is also likely to reduce the cost prize of insect derived products, thereby increasing the economic viability of the sector.

Insect farming for better nutrition, health and livelihoods in Africa

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Farming insects has just within the past decade emerged as a novel animal production sector in Africa. Successful upscaling of insect farming in Sub-Saharan Africa has the potential to contributing to more sustainable food systems, to improving food security and nutrition, and generating income across the value chain. A non-systematic survey has indicated insect farming activity in a majority of countries on the continent. However, the insect sector is still scattered and remains to consolidate as a mainstream production sector. Kenya, Uganda and Ghana are among the countries with pioneering insect farming and innovation in insect products. Building on previous research, we have identified key barriers for adopting and scaling up insect farming to be lack of knowledge and access to information about insect farming, and lack of awareness of the nutritional value of insects and processed insect products.

In the HEALTHYNSECT research project, we are investigating the drivers for small-scale farmers to initiate insect farming, exemplified at three sites: Farming crickets (*Acheta domesticus* and local species) in Kenya, grasshopper (*Ruspolia differens*) in Uganda and palm weevil (*Rhynchophorus phoenicis*) larvae in Ghana. By conducting a multi-site cluster-randomized intervention study of introducing different insect farming systems, we investigate the direct effects of overcoming barriers for insect farming by providing training and a start-up kit, alone or in combination with stimulating the demand for insects by introducing a healthy insect-based product for children. We will assess the impact on the perception and willingness to take up insect farming and to consume insects on a regular basis. We also assess the impacts of insect-based products on gut health in small children.

The interdisciplinary collaborative research will contribute to provide a framework for policies to overcome barriers and stimulate the upscaling of insect farming in Africa.

Insects, sustainability and more

Mark Benecke

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Introduction to Mark Benecke:

He works in the forensic field for over twenty-five years. He set up forensic DNA labs in the Philippines and Vietnam, was employed at the Chief Medical Examiner's Office in Manhattan, can often be found training students from Colombia to Cologne and is Germany's only Certified & Sworn in Expert for Detection, Collection and Examination of Biological Stains (Öffentlich bestellter und vereidigter Sachverständiger). He did his Ph.D. at the Inst. for Legal Medicine in Cologne, took part in police trainings all over the world (including the FBI and the Body Farm) and wrote forensic books, including books for kids, as well as numerous scientific articles.

The older he gets, the more he realizes that Conan Doyle's Sherlock Holmes already did much of what we think we discovered first.

Lecture

Topic 1–5

Would an oat milk producing farm see a (financial) bonus to raising black soldier fly on its residues?

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The production of Oat Milk produces high-quality residues that are often sold as pig feed. A strong sustainability case can be made for using the oat fiber residues (OFR) to raise Black Soldier Flies Larvae (BSFL) instead, as this would support a more circular production system. This thesis sought to investigate whether such an arrangement would be more profitable for the Oat Milk Producing Factory (OMF), or, in other words, if evidence of a 'BSF Bonus' could be found.

This was done by creating a financial model (within the context of a Bulgarian farm) to model both a 2.5 Ton/Day output and 5 Ton/Day output OMF, along with a BSFL facility scaled to digest the OFR produced by each OMF. The model also allowed the simultaneous analysis of a 30 Ton/Day Food Waste Processing Facility (FW), which operated under different assumptions due to size, purpose and output. These were then assembled into 5 scenarios of equal length to allow comparison, including the question of whether there was a 'BSF Bonus' for the OMF that used BSFL compared to those that simply sold their OFR.

A Cost-Benefit Analysis (CBA) of their cashflows was performed giving a discounted Net Present Value (NPV) for each scenario. A sensitivity analysis was then performed to identify which variables disproportionately affected the final NPV of the scenarios. These were then used for a Monte Carlo analysis in Crystal Ball; running the models many times (n=100k) with random values for these variables selected simultaneously. The random variables were probabilistically selected according to defined probability distributions, of various shapes, operating within specified likely ranges according to argument and research.

Despite using a high discount rate, all scenarios yielded consistently positive NPVs. There was, however, up to a 20% chance that there would be no BSF Bonus for the OMF. This was due to the reliance on consumer-level sales, of which demand and price were highly uncertain. This could be balanced by a better valuation of the intangible benefits. The large FW facility was consistently profitable, even when consumer-scale sales were removed, suggesting benefits of scale.

Monitoring of *Tenebrio molitor* pupae based on Region Based – Convolutional Neural Networks (R-CNN)

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Due to the growing word population, alternative food sources must be explored. Mealworm larvae (*Tenebrio molitor*) offer such an alternative source, instead of traditional food like beef or fish. The European Food Safety Authority published a paper about the safe use of mealworms as a novel food early this year. The only obstacle is the formal admission of the European Commission, which should follow soon. Considering this development a growing demand for mealworms can be expected. To face the higher demand the rearing process must be automated.

To increase the food conversion ratio (FCR), it is important to encounter the right harvesting time to avoid weight loss during the pupation phase. One approach for this task is a neural net based optical monitoring system to detect pupae. Also for reproduction purposes a detection of pupae could be helpful.

Neural networks offer some advantages over classical image processing. Neural networks are able to handle inhomogeneous images with overlapping pupae. Furthermore, partially covered pupae can likewise be detected.

In this case a Region-Based Convolutional Neural Networks (R-CNN) was used. The algorithm creates about 2000 image regions, which are generated by a selective search algorithm. These regions are then classified by the R-CNN. At last it marks desired objects with a bounding box.

The network and all pre-processing steps were implemented in python. In a first approach, the true positive rate was 52%, with 39 out of 75 pupae detected. The precision of the net was 81%, with 9 objects falsely detected as pupae. These first results are promising for detecting pupae in an industrial rearing capacity. Due to the rather small database with only 160 images containing 1077 pupae, a proper learning is hardly possible. A better result can be expected if more data is used to train the network. This can be alternatively done by altering the size, rotation or stretching of images.

Use of grid to improve egg laying efficiency of *Tenebrio molitor* beetles

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Although mealworms have been cultivated as feed for pets and zoo animals for some time, this industry is still in its early stages. Whereas the production of conventional farm animals can draw on years of experience, there has hardly been any research focused on the optimization of breeding and reproduction of mealworms. During their life cycle, the mealworm *Tenebrio molitor* passes through 4 stages: egg, larva, pupa and beetle. For reproduction, beetles are placed in containers with feed where they can lay eggs. After a specified time the beetles are removed and the eggs in the container can develop into larvae. Density and egg laying duration negatively influence the number of produced mealworms per beetle. This is partly due to cannibalism as using an egg laying grid reduces this negative influence of density and egg laying duration. Reproductive success has so far always been assessed by counting the number of offspring several weeks after the beetles laid eggs. As a result, the number of eggs laid and their hatch rates are not known. Therefore, it was investigated how eggs can be separated from their substrate. This allowed to determine how egg laying and hatching are influenced by density, egg laying time and the use of an egg laying grid.

The potential of data in an industrial insect plant

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Industrial insect production with automated systems has become a viable business opportunity. In these large-scale plants, it is very important to produce safe products with consistent high quality, while maximizing the yield. To optimize the plant performance over the entire lifetime, the ability to continuously monitor production data is key, because it allows to take potential corrective actions in time.

The basis for a data-driven plant optimization is data transparency. This means that (i) the machines and instruments of an insect plant get connected, (ii) all data are stored on a central platform, and (iii) live and historical data are visualized for different stake-holders. In this presentation, several visualization options are presented. Dashboards that show key operational parameters or performance indicators and monthly reports summarizing machine performance are basic options to represent data for better process understanding, but also for digital assistance with regards to audits. More advanced visualizations can be achieved with replay functions of the automation system, allowing to display the state of production lines at any given point in the past. This helps to achieve maximized uptime of the lines through faster diagnosis in the case of any problems.

Going one step further, thorough analysis of the data lake empowers to implement even self-optimizing process steps in the insect plant. This is meaningful for key steps with a direct impact on plant profitability. Some examples will be presented, in which specific sensors allow to safely tighten tolerances on a key parameter leading to improved yield as well as reduced energy and waste.

It can be concluded that an effective data strategy and state of the art tools are already crucial during the start-up of an insect plant, since they help to achieve the full production capacity within shorter time. Moreover, transparent process insights will support operators to move quickly along the learning curve during production allowing continuous improvements of key performance indicators, thus making an insect plant even more financially attractive.

Determination of moisture, protein and fat content in living mealworm larvae (*Tenebrio molitor*) by near infrared reflectance spectroscopy (NIRS)

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The yellow mealworm (*Tenebrio molitor*) is an edible insect and, due to the frequency of consumption, a promising candidate for breeding and production on industrial scale. NIRS emerged as a fast technique for quantitative determination of several nutritional parameters simultaneously and is used for quality and process control of food and feed.

In our previous studies, we could see that nutritional composition of the feeding substrate has an influence on insect larval composition. In order to ensure a balanced and targeted feeding or to achieve a desired larval composition, it is essential to monitor and regulate larval feeding.

The present study represents a new approach to analyze changes in the nutritional composition of *T. molitor* larvae using NIRS combined with multivariate analysis. Living larvae were scanned with a near infrared spectrometer using wave lengths from 1100 to 2100 nm. Different feeding substrates with varying moisture, protein and fat content were tested and the influence on larval composition was measured. Spectral curves were correlated with larval moisture, protein and fat content analyzed by analytical standard methods. A calibration was developed, using MATLAB and PLS toolbox, with modified partial least squares as regression method. The NIR spectra were influenced by composition of feeding substrate and larvae, since the absorbances of the various larval groups differed greatly. Nutritional content of the feeding substrate had also a significant influence on the development and weight gain of larvae. As a significant result, differences in larval composition could be determined, depending on the feeding. With respect to wet weight of the larvae moisture content varied from 60 to 74%, protein content from 16 to 24% and fat content from 4 to 10%.

This investigation shows, that with a non-invasive NIRS online monitoring, the composition of insects can be continuously recorded and evaluated so that specific feeding can be carried out in the course of larval development. In addition, different batches can be produced, tailored to the target organism such as chicken, pork or fish and their nutritional requirements, which are to be fed with insect larvae.

Feasibility of small-scale BSFL composting in the EU

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Organic wastes have a low monetary value but are the foundation of an emerging sector: Fly larva composting. Moving away from and finding alternatives to linear feed production is crucial to achieve a circular economy in the feed sector. Producing larval meal from organic waste is one possible way forward. This would reduce the need to import soybean and fish meal, while increasing the value of organic side streams. Furthermore, live larvae could serve as a feed amendment for layer hens, while at the same time act as an environmental enrichment that encourages hen foraging behavior. Thus an increased price could be justified through improvements in animal welfare and environmental sustainability when using waste-reared fly larvae in livestock production.

As the fly larvae composting technology advances, the possibility of achieving feasible circular feed production systems becomes more realistic. In spring 2021, a project financed partly by Vinnova and Axfoundation diverted around 10 tons of food industry waste, from vegetable cuttings and returned bread that was converted into 1,7 tonnes of black soldier fly larvae and 2 tonnes of compost. The fly larvae composting process took place in modified shipping containers that are mobile and can be placed at the waste source. Ventilation can be regulated to the amount of waste treated and the maximum treatment capacity is 190–260 kg/d (10–14 kg/m^2, d) depending on the waste type treated. The larvae were further processed and pressed into feed pellets for rainbow trout, while the compost was used as soil amendment for a permaculture garden and on private properties.

In this presentation we illustrate the potential of resource recovery using fly larvae composting, but also highlight the economic struggles a small-scale insect business may face. We will present our current findings and will compare the revenue from the small-scale experiment to the current market prices and show why protein production should include waste treatment.

Isoenergetic, isonitrogenous, and semi-purified diets for lipid requirement determination in *Hermetia illucens* larvae

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Some insects are able to efficiently convert waste into a resource, and the black soldier fly (BSF) is one of the most promising species. To make high-performance BSF waste bioconversion, it is necessary to determine the larvae nutritional requirements. The aim of this study was to evaluate the optimal substrate lipid level for larval growth. Five isoenergetic, isonitrogenous, and semi-purified diets with increasing lipid levels (1%, L1; 1.5%, L1.5; 2.5%, L2.5; 3.5%, L3.5; 4.5%, L4.5) were tested (100 larvae/box; 6 replicate boxes/diet). The Gainesville diet was applied as environmental control and, subsequently, excluded from the statistical analysis. Larvae growth was determined at 10, 14 and 18 day-old (30 larvae/box), while survival rate was calculated at the end of the experiment when 40% of prepupae per box was identified. The prepupae weight was recorded on 30 individuals per box. Data were analyzed by means of Kruskal-Wallis (IBM SPSS Statistics V20.0.0, $p<0.05$). At 10 day-old, the L4.5 larvae showed the greatest weight when compared to the other treatments ($p<0.001$). On the contrary, in the second sampling (14 day-old), the diets L1.5, L2.5 and L3.5 were not significantly different from the L4.5, while the L1 showed the worst weight ($p<0.001$). The L1 larvae recorded the lowest weight when compared to the other groups even in the last sampling ($p<0.001$). Furthermore, the average weight of the L2 treatment was lower than the L3.5 (0.229 g and 0.249 g; respectively). Larval survival was not affected by the dietary treatment ($p>0.05$). The L1, L1.5 and L2.5 prepupae weight was lower than the L3.5 group ($p<0.001$), while the L4.5 showed an intermediate result ($p>0.05$). In conclusion, substrates lipid levels of 1% or less have negative effects on larvae growth. As regards prepupae weight, the lipid percentage of 3.5 seems to have positively influenced the prepupae size. This work was funded by Fondazione Cariplo Project "Circular economy: live larvae recycling organic waste as sustainable feed for rural poultry" (project acronym, Cellow-Feep).

Bioconversion of chicken manure by housefly larvae (*Musca domestica L.*); larval performance and substrate conversion in relation to sterilization and carbohydrate addition

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The larvae of the housefly can be reared on manure and used as animal feed. Larvae can utilize proteins and easily digestible carbohydrates and have a largely unknown relation with manure microorganisms. Our study hypothesis was that larvae compete with microorganisms for easily digestible carbohydrates such as starch. This was tested by adding starch, digestible by both larvae and microorganisms, or fructo-oligosaccharides (fos), digestible only by microorganisms, to unsterilized or heat sterilized fresh chicken manure (diets: CMstar, CMfos, sCMstar, sCMfos).

A housefly culture was maintained in a climate room (28 °C, 70% RH, light-dark 16:8). Larvae were reared on wheat-bran-milk powder based substrate and adults were fed granulated sugar and milk powder ad-libitum. Eggs were collected through oviposition on black cotton socks suspended in milk. Diets and pure manure controls (CM, sCM) were inoculated with housefly eggs (6 replicates, 1250 eggs / replicate) by weight and larvae were harvested after five days by flotation. Total larval mass and survival were determined, as were nitrogen and DM content of larvae, diet, and residues.

The highest yield (9.7 g) and heaviest larvae (13.2 mg) were on sCMstar, followed by sCM (7.2 g and 8.1 mg). Both CMfos and sCMfos had minimal yields and larval weights (0.3 and 0.2 g, 2.9 and 1.7 mg) with CMstar intermediate (3.3 g and 7.5 mg). Survival differed between diets, sCM and CM averaged 70% (898 and 886 larvae), sCMstar 57% (736 larvae), CMstar 33% (356 larvae) with 9% for CMfos and SCMfos (106 and 100 larvae). DM bioconversion decreased from CM (3.5%), sCMstar (3.1%), sCM (2.6%), CMstar (1%) to CMfos and sCMfos (0.1%). Nitrogen bioconversion was highest on sCMstar (9.9%) and CM (9.3%), lower on sCM (6.6%), CMstar (4%) and the lowest on CMfos and sCMfos (0.5%). The results concur with the study hypothesis but indicate also that sterilization and carbohydrate addition might not be ideal for optimization of chicken manure as larval diet.

Results of long-term selective breeding of *Hermetia illucens* for industrial applications

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In the last ten years, *Hermetia illucens*, the black soldier fly (BSF), has become one of the most farmed insect species. Interest in the species stems from the ability of the larvae to convert low-grade organic materials into high-value food, feed, and technical products. Despite the inherent efficiency of this species, significant improvements in performance are possible. One means to improvement is selective breeding, which has been widely applied in other livestock species.

Selective breeding, which has been practiced for thousands of years, relies on mating animals with desirable traits to concentrate these traits. This contrasts with gene editing approaches. Traits of interest in livestock include faster growth, higher finishing weight and lower feed conversion ratios. A successful selective breeding program is a considerable scientific accomplishment, and furthermore if the results are applicable in an industrial setting the effect on the economics of production and its environmental impact can be considerable.

Literature on BSF has explored genetic variation in the species, genetic plasticity (BSF) and reported on preliminary insights from sequencing. To date, there have been no public research results reflecting a focused, multi-year selective breeding effort. In 2019, Protix and Hendrix Genetics began selective breeding for increased body weight. The research presented summarizes the outcomes of this multi-year program. Over the course of the selection program, significant increases in relevant parameters have been achieved and will be reported. Larval performance improvement of selected line over base population line has been evaluated for standard measures on larval weight, total yield and nutritional composition. The means used to achieve improvement remain confidential, but the research outcomes are relevant in their own right as they provide an indication towards the potential that selective breeding may play in the cultivation of BSF. This may stimulate further research interest in this topic.

Automated, modular systems for rearing *Hermetia illucens* larvae – design considerations and sustainability

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The black soldier fly, *Hermetia illucens*, has received much scientific attention in recent years due to its ability to consume even challenging organic residue streams, effectively reducing their mass and producing valuable proteins and lipids in the process. Thus, it can be and already is being used to utilise agricultural side streams and wasted food, mitigating environmental impacts and providing valuable resources.

One challenge for implementation of this technique is utilizing residues from many decentralized sources without excessive labour costs. Thus, the aim of this study, within the UpWaste project, is to develop automated and modular systems for rearing *Hermetia illucens* Larvae on a range of agricultural side streams.

The project is carrying out rearing and processing trials as well as biological hazard analysis to assess the suitability of different common residues, with or without additional preprocessing, for rearing of black soldier fly larvae and their safety in the context of organic material recycling.

An innovative system for automatic feeding, handling and harvesting of larvae and side products housed within a standard shipping container for easy preassembly and relocation is under development. Based on this design and experimental data on the growth performance of larvae, energy and material balances will serve as a basis for economic and ecological life cycle assessment. In this manner, it will become apparent whether the UpWaste system can be an economically and ecologically sustainable option for waste treatment.

Bioreactor-based mass cultivation of insect larvae

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Insects are a noteworthy sustainable source of animal protein for food and animal feed. The larvae of black soldier fly (*Hermetia illucens*) convert low-value biowaste to insect biomass very efficiently (1 kg dry mass can provide up to 1 kg fresh larvae). Insect protein, however, faces a heavy price competition against fish and soy protein in the animal feed market. Consequently, insect rearing, regarding both the investments and the operation costs, must be very cost-effective. We believe that Entoprot's highly efficient bioreactor technology can significantly cut down the costs of insect rearing.

Today's golden standard in insect rearing means vertical farming of insects in stacked boxes. This cultivation principle however requires expensive automation and big investments. Additionally, the quality of the produce can be inconsistent, as the conditions often vary from box to box. For optimal efficiency, big insect protein factories must be located close to ample raw material sources. Major companies, even non-profitable ones, can easily outcompete out smaller companies which have a better potential to manage the local raw material sources.

Entoprot 's scalable bioreactor-based technology is affordable for companies of all sizes. Our rolling drum bioreactors are equipped with support structures which prevent larvae being crushed. We have obtained 70% fill volume, equivalent to 420 kg fresh larvae per cubic meter per a single cultivation. As the larvae, transferred into bioreactor as minilarvae (neonates pre-cultivated for two days), can reach a harvest weight already in 7 to 10 days, bioreactors can easily provide 1000 to 1500 kg fresh larvae per cubic meter per month.

Entoprot Ltd presents a completely new and highly efficient technology for cultivation of insect larvae. The productivity per foot area can be 5-fold higher in comparison to vertical farming, and due to inbuilt automation, the need for manual labour is minimal. We believe that 1) this system can become a new standard in rearing insect larvae for all size companies and 2) our technology helps to bring the insect meal price below the market price of fish meal.

Boundary conditions in insect engineering (from air humidification to the fractionation to the end product)

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In recent years, a new insect-related industry has been born. There we have the same requirements or influencing factors around this new production field compared to other industries. That means if we have a new constructed plant at first we have a look at the relevant emissions like particle and smell in the air as well as temperature and water recycling. Many parameters work together and have a great influence on the end product especially if we talk about hygiene. Besides the black soldier fly, we can find very often the yellow mealworm. If we look to the live cycle, the growing period can be approx. 80 days depending on the substrate. With a growing temperature between 26 °C and 30 °C and an air humidity of approx. 60% the growing conditions for bacteria and moulds are very good.

Depending the growing conditions, we have to look at the fractionation process. Especially for the feed industry, we need always the same quality of the protein carrier starting with the inactivation process. In our studies, we observe the material composition (soluble protein, AA-profile, hygiene) after inactivation and the following processings like wet or drying fractionation.

Purines in edible insects and their suitability for people suffering gout

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Edible insects exhibit potential as a good food source for the increasing human population. They are a good source of protein, the digestibility of which is comparable to certain commercial protein sources. However, protein-rich foods are also a source of purines. They are aromatic heterocycles composed of carbon and nitrogen including adenine and guanine, which participate in DNA and RNA formation. The final product of purine metabolism in the human body is uric acid. Frequent and excessive intake of purine-rich foods enhances the levels of uric acid in serum, which in some people results in gout and could be a risk factor for cardiovascular and kidney disease as well as metabolic syndrome. Therefore, for approximately 1–4% of the western population suffering gout, insect consumption may be problematic.

The contents of four purine derivatives (adenine, guanine, xanthine, and hypoxanthine) and their common metabolite – uric acid – were analyzed by RP-HPLC with UV detection in males and females of three insect species: house crickets (*Acheta domesticus*), desert locusts (*Schistocerca gregaria*), and discoid roaches (*Blaberus discoidalis*). Total purine content (in g/kg DM) decreased as follows: house cricket male (6.96 ±3.52) > desert locust female (6.22 ±0.65) > house cricket female (6.01 ±3.16) > desert locust male (4.95 ±0.25) > discoid roach male (3.10 ±0.80) > discoid roach female (1.17 ±0.56). Uric acid content decreased as follows: discoid roach > house cricket > desert locust. Species and – in some cases – sex had significant impacts on purine derivative and uric acid contents in edible insects.

Our results provide new data regarding the content of purine derivatives and uric acid in selected edible insect species and confirmed that these insects constitute a rich source of purines and uric acid, the contents of which varied depending on species. Moreover, the results indicate that sex is also a factor affecting purine and uric acid content – at least in certain edible insect species. The obtained results indicate that patients suffering gout should be cautious about excessive and daily consumption of edible insects and the products made therefrom.

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

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The insect industry or industrial insect sector is not new, but presently developing on a fast and high level. Current challenging tasks are the large-scale rearing and breeding which can be compared to a commercial animal rearing facility. In comparison to this the technology readiness level of the processing part is already high. Evolving market needs for pet food or aqua culture will lead to more customized processing concepts.

Beside these theoretical design aspects of processing insects also several practical points enter the game, like finding a balance between price and quality.

With a history of over 8 years in the insect industry REINARTZ developed numerous dry processing solutions for black soldier flies, mealworms & crickets.

Based on the insect farmer's figures of wet larvae capacity, harvesting strategy and shift plans the dry process will be designed. Evaluation of the drying technology is one key point of a sustainable and successful insect processing. The other essential topic is that separation with screw presses achieves traditionally high defatting values and leads to high protein rates. Typical results are around 8% of residual fat and a protein rate exceeding 60%. In order to meet market demands in regard to digestibility and solubility the process temperatures can be precisely maintained.

The trend towards decentralized rearing and processing facilities leads to small specialized units, flexible but focused on high quality products. Therefore, the dry processing is the blueprint for a successful business case.

Benchmarking insects suitability in food supply chains under crisis conditions

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Wars, extreme weather conditions and pandemics can impact global food security by affecting import and export. This study investigates the capability of food supply chains of insects relative to chicken meat under such crisis conditions. Comparisons were also made with cultured meat, algae, mycoproteins, and plant-proteins. For insects *Tenebrio molitor* was studied, plant proteins were represented by pulses. An autarky situation was assumed in which import and export is absent.

Based on literature four main factors were defined that influence a foodstuffs capability under crisis conditions:

1. efficient production characteristics to ensure food availability
2. good intrinsic attributes to ensure food utilization
3. a production and distribution that relies on an efficient supply chain to ensure food access, and
4. a feasible conversion from conventional to crisis food production.

Assessment of the criteria for production characteristics, namely protein yield (g/m^2/day), land-, water-, and energy-requirement, showed that *T. molitor* has favourable production characteristics in comparison to chicken, while the other alternative protein sources lacked on 1–3 criteria.

T. molitor was, like cultured meat, similar or more suitable than chicken for intrinsic attributes with respect to safety risks, nutritional properties, sensory properties and shelf life. Although consumer acceptance was limiting as such, it could naturally increase under crisis conditions. Algae had the lowest suitability overall.

When analysing the efficiency of the supply chain, expressed as the N° of actors, access to raw materials, and access to consumers, *T. molitor* and pulses were favourable compared to chicken. Cultured meat and algae have more complex supply chains.

Further, *T. molitor*, pulses, and algae were identified as the sources with the highest feasibility of conversion, also when compared to chicken, as the knowledge and technology required for production was low. However production of cultured meat and mycoproteins required much more advanced knowledge and technological operations in the production process.

Overall insects have – relative to other novel protein sources – high potential as food under crisis conditions and could be complementary to chicken meat.

Agro-food leftovers as insect feedstock for producing carotenoid-rich *Hermetia illucens*

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Nowadays, the insect mass rearing production is promoted not only for the insect nutritional value, but also for their ability to bioconvert agro-food leftovers, which otherwise would be discarded, into high valuable proteins and fats. Previous studies have already demonstrated the potentiality of tailoring a promising diet by mixing agro-food leftovers in order to maximize the feed conversion rate, as well as to improve the final nutritional value of *Hermetia illucens* larvae (BSFL).

In the present work, we evaluated the potential production of carotenoid-rich BSFL by rearing them on specific agro-food leftovers rich in α- and β-carotene, lycopene, lutein and zeaxanthin. The results showed that the carotenoid concentration in BSFL was strictly correlated with the carotenoid content in the insect growth media. Among the different substrates, tomato and carrot leftovers allowed to obtain BSFL with a total carotenoid content 230% higher than the amount detected in the BSFL grown in the control diet. Furthermore, we demonstrated that the killing method plays a key role in preserving the BSFL carotenoid content, with dry heating (100 °C for 2 minutes) resulting in a significantly higher lycopene, α- and β-carotene content compared to blanching (100 °C for 40 seconds) and freezing (–20 °C).

These results clearly support the exploitation and valorisation of agro-food leftovers for the production of BSFL naturally rich in carotenoids with enhanced bioactive properties, to be used for specific feed and food purposes.

This work was done in the framework of the project “FLIES4VALUE” co-funded by POR FESR, Program 2014–2020 (Italy), Axis 1, Action 1.2.2 Call for Research Grouping.

Fractionation of lesser mealworm (*Alphitobius diaperinus*) and characteristics of a cream layer

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Fractionation is widely used to separate insect biomass into fractions with varying composition, physicochemical characteristics, and techno-functional properties. One approach is wet fractionation via solubilization of insect proteins with subsequent centrifugation and separation of insects into pellet, supernatant, cream layer, and oil fractions. The extractability of soluble proteins can be enhanced through enzymatic hydrolysis that also leads to alterations in fractions ratio and changes in protein functionality.

The cream layer is a minor fraction naturally formed upon fractionation that mainly consists of proteins and oil. This study aims at investigating the main physico-chemical characteristics of the cream layer, its stability, and reconstitution ability. The cream layer was obtained from blanched lesser mealworm (*Alphitobius diaperinus*) larvae during aqueous extraction with and without addition of the proteases Alcalase and Neutrase. The yield and protein content of the cream layer were significantly lower for the cream layer produced by the enzyme-assisted method. The cream layer after hydrolysis was distinguished by the abundance of proteins smaller than 20 kDa. Evaluation of long-term stability using confocal microscopy and Mastersizer showed little changes in cream microstructure after 56 days of storage at 4 °C, that was in accordance with relatively stable values of oil droplet size. An accelerated stability test demonstrated that only less than 20% of the cream was destabilized after 59 days of storage at 4 °C. Re-constituted cream obtained from freeze-dried and spray-dried cream layerobtained after hydrolysis showed similar particle size distribution to fresh cream. Other characteristics of reconstituted cream, such as solubility and stability under accelerated conditions were significantly affected by the presence of enzymes in the production of the cream.

Thus, the cream layer formed during fractionation of lesser mealworm represents a highly stable oil-in-water emulsion stabilized by mealworm proteins. It can be used as a food ingredient for emulsion-based foods, and freeze-drying and spray-drying were determined to be promising methods to extend its shelf-life.

Target marketing on edible insects' business

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Edible insects are highlighted as a future protein source having high sustainability. Despite the big attention in western countries, edible insects' market in Japan is limited so far. In Japan, we have a food culture of eating locusts and bee larva. However, the traditional food culture is now disappearing with the westernization of our diet. As a result, many people have a feeling of disgust or resistance to eating insects due to food neophobia. Powder-formed product will be a key solution to avoid psychological resistance to insects.

To reveal the profile of early adopters of insect food, questionnaire regarding the food preference and consumer behavior was carried out. Segmentation analysis was applied to the target group (n=500) who buy cricket flour and control group (n=966).

Factor analysis resolved a six-factor solution. Each of these factors has a distinctive grouping of questions that can be associated with food preference and consumer behavior (Factor 1: interest in production area, Factor 2: neophilia, Factor 3: neophobia, Factor 4: willingness to pay, Factor 5: health awareness, Factor 6: conformity). Following to the factor analysis, a non-hierarchical cluster analysis was performed by using the factor scores, and respondents were grouped into 3 segments (Segment 1: majority synching, Segment 2: conservative, Segment 3: curious). Significant difference was found in the proportion of each segment between target and control groups. Curious segment accounted 60% of target group while 10% of control group. The respondents who grouped into the Segment 3 (curious) showed higher weight for Factor 1 (interest in production area), Factor 2 (neophilia), Factor 4 (willingness to pay), and Factor 5 (health awareness). These profiles would be the key directions on development of edible insect products. Targeting strategy is critical to create an initial market. From this context, segmentation analysis provides valuable solutions.

The potential of dry fractionation for insect processing

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Due to the global need for sustainably produced protein, novel protein sources like insects are being explored. Currently, wet fractionation is often used to obtain protein-rich insect ingredient fractions, although it is very energy intensive and consumes a lot of chemicals. Dry fractionation could be a more sustainable approach while also providing ingredients with retained native functionality as was shown for plant products. In dry fractionation, methods like air classification, sieving, and electrostatic separation are used to separate compounds based on factors like particle size, particle density, and tribo-electric charging properties. The aim of this study is to evaluate the use and potential of dry fractionation for insects. The results are compared with shellfish waste, as chitin removal also plays a role in these products, and also with meat and bone meal, fish meal, and plant products. Plant products were chosen as dry fractionation is more common for these sources. The reviewed studies of both insects and the other animal products predominantly focus on enrichment of desired compounds, or fractions with distinct functionalities. Depending on the dry fractionation process and settings, protein enrichment of up to 5% was found, but the process was not optimised. Contrary to plant proteins, air classification of insect powders caused protein enrichment in the coarse fraction. This is caused by the protein that is, together with chitin, present in the tougher exoskeleton. After dry fractionation, the obtained fractions differed in amino acid profiles, solubility, and sensory characteristics. As of now, very limited in-depth studies are available on processing conditions linked to material properties, composition and functionality. Furthermore, the studies focus on proving the principle, not on optimising the process. For dry fractionation of insects, optimisation can be found in the entire process chain. Overall, these results show that dry fractionation is a promising technique for insect processing. However, more research is needed in this area.

Why black soldier fly larvae protein determinations bug(ged) researchers

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Edible insects have the potential to deliver a significant contribution to the protein transition that is on the horizon in Europe. However, a broad discussion currently exists in the literature regarding insect protein analyses. The main difficulties and uncertainties on this topic are the inconsistent use of analysis methods and nitrogen-to-protein conversion (*k*P) factors. While the Kjeldahl and Dumas method are both applied for quantifying the nitrogen content of edible insects, their result is an inherently different nitrogen fraction. More specifically, the Dumas method determines the total nitrogen content whereas the Kjeldahl method measures only organic nitrogen. Thus far, no correlation between these methods has been established for insect matrices, which is a major uncertainty.

In order to convert the nitrogen content into a protein content, the so-called *k*P factor is applied. In general, 6.25 is used as *k*P factor to calculate the crude protein content, which assumes that all nitrogen originates from proteins and that their nitrogen content equals 16 g/100 g protein. Despite the efforts that already have been made towards more accurate *k*P factors, calculations of these factors were based on only one sample, while the chemical composition of insects varies with life stage, rearing substrate and conditions.

In the present study, a correlation between Kjeldahl and Dumas and a new *k*P factor has been established by using a broad variation in black soldier fly (BSF) larvae samples. Moreover, their nitrogen distribution was assessed as well after accurate chitin analyses.

A highly significant linear correlation was found between the results of the Kjeldahl and Dumas method, having a slope of 1.009 and an intercept of –0.008. Consequently, both methods were deemed interchangeable for BSF larvae.

From the different BSF larvae samples, a practical, more accurate and robust *k*P factor of 4.43 was obtained using amino acid data.

Concerning the chitin content, the average of all BSF larvae samples was 5.95 ± 0.86 g GlcNAc/100 g dry matter and no correlation with the *k*P factor was observed. As for the nitrogen distribution of the samples, it was found that the contribution of nitrogenous compounds other than protein and chitin was not only high but also prone to variation (12 – 30% of the total nitrogen content).

The first novel Food Authorisation for Insect: a historical and necessary milestone for the whole industry

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Insects as food are deemed very promising as a solution to address future food challenges. However, the development of this sector within the European Union (EU) was limited in the past years, especially by a lack of official authorisation.

The Regulation (EU) 2283/2015 of the European Parliament and of the Council on novel foods which came into force the 1st January 2018 clarified the marketing of edible insects within the EU.

However, it was not until the 1st June 2021 that the first official authorisation to market edible Insects within EU was granted to Agronutris, a European pioneer of insect production as protein source. The obtention of this authorisation is a historical and necessary milestone for the development of the edible insect market and the promotion of insect integration in the diet of Europeans.

The authorisation obtained by Agronutris required the filing of a Novel Food dossier. In this presentation, the content of the application dossier will be detailed (production process, compositional data, toxicology, allergenicity). Then, the filing process will be described from the first step of required data identification to the obtention of the authorisation of marketing the Novel Food upon European Food Safety Authority (EFSA) evaluation. Furthermore, the specificity of this first insect Novel Food will be highlighted in terms of labelling, conditions of use and data protection. Finally, the input of this first authorisation to the whole insect industry will be discussed.

Safety of black soldier fly (*Hermetia illucens*) larvae reared on different biowaste substrates

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Black soldier fly larvae (BSF, *Hermetia illucens*) can convert low quality organic waste into protein-rich ingredients for food and feed. The underutilized biowaste streams could be a source of valuable nutrients, however, they could also contain chemical residues. For example, antibiotics and antiparasitic drugs are regularly found in pig or chicken manure and could be present in slaughterhouse waste. Pesticides residues can be found in organic waste of for instance vegetal origin. Under current European law, it is not permitted to use biowaste substrates that contain animal products or manure for insect rearing, therefore it is needed to complete the necessary food and feed safety data to enforce possible legalization.

This study aimed to investigate the effects of the presence of chemical contaminants in biowaste substrate on black soldier fly larvae, including their growth and survival, as well as on the presence of residues in the larvae. Seven days old larvae were reared on chicken feed (reference diet), catering waste, liquid pig manure mixed with silage grass, solid pig manure, mushroom feet, slaughterhouse sludge and organic wet fraction of household waste for one week. Insects were reared on the waste streams in a small scale experiment (50 g substrate) and larger scale experiment (10 kg substrate). Results of insect growth and analysis of the presence of chemical contaminants such as heavy metals, veterinary drugs, pesticides and acrylamide in the substrates and larvae will be shown, as well as possible differences due to the scaling of the experiments.

When rearing BSF larvae on biowaste substrates, the possible presence of some chemical contaminants such as heavy metals and veterinary drugs should be controlled to ensure optimal insect growth and safety of the insect products.

Effects of pesticide residues on insects reared for food and feed

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Insecticide residues may remain in feed materials that are intended to be used for insect rearing. Maximum residue limits (MRLs) have been set for such substances in the EU – but low concentrations that comply with MRLs could still affect insects reared for food and feed. In this study we aimed to investigate effects of insecticide residues on insect growth and survival, as well as the possible accumulation of the substances in the larvae. A series of experiments on black soldier fly larvae (BSFL, *Hermetia illucens*) and lesser mealworm (LMW, *Alphitobius diaperinus*) was performed. Tested substances included: chlorpyrifos, propoxur, imidacloprid, spinosad, tebufenozide, fipronil, pirimiphos-methyl, cypermethrin, and piperonyl butoxide (PBO). We found significant effects on growth and survival when the two species were exposed to two of these insecticidal substances at concentrations equal to the MRL. For example, up to 72% mortality was recorded for BSFL when exposed to spinosad. Bioaccumulation of tested substances was not observed, but incomplete mass balances suggest that metabolization has occurred to some extent. LMW appear to be more tolerant to tested insecticides than BSFL. These findings call for lowering MRLs in regulation of feed materials used for insect rearing. Insect rearing companies are recommended to monitor incoming feed materials for pesticide residues. More research is warranted on a wider variety of insecticides, resistance mechanisms, and sublethal effects.

Inoculation experiments with food pathogens during insect rearing and during heat treatment of frass

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In microbiology, protocols are in use to inoculate a micro-organism in a certain niche or in/on a matrix and then study its behavior under the conditions prevailing in that niche or matrix with respect to available nutrients, intrinsic and extrinsic properties and background microbiota. When performed with food pathogens, this type of experiments can contribute to risk assessments and evaluation of microbiological safety in the insect sector. Pathogens can be inoculated during insect rearing to study their transmission potential to the insect, but they can also be inoculated after rearing on harvested and killed insects or in frass to study their survival during processing. Whatever the aim of the inoculation experiment, a lot of factors may influence the conclusions, and hence potentially (legal) criteria prescribed to the sector later. Therefore, a careful and realistic design of such trials is extremely important.

In this presentation, an overview will be given of the choices that need to be taken (and that may affect the results) and the difficulties that can arise during inoculation experiments, based on ample experience with such trials in our research group. Choices relate to the selection of strain(s) used as target organism(s), the inoculation procedure, the inoculation level(s) considered, the number of replicates in and repetitions of the whole experiment and the inclusion of proper controls. As to difficulties, the focus will be (i) on how to deal with the background microbiota that often impedes specific monitoring of the inoculated strain(s) and (ii) on possible airborne transmission from inoculated to control samples during rearing. Example results obtained in several projects will be discussed for inoculation tests during rearing of mealworms or black soldier fly (BSFL) with *Salmonella* or *Staphylococcus aureus*, as well as during heat treatment of BSFL frass (1h at 70 °C) after inoculation with *Clostridium perfringens* and *Salmonella*. Conclusions should be formulated in a correct way with respect to possible colonization, reduction or killing of a pathogen. Also caution is needed when comparing results between different authors and generalizing conclusions, since many aspects relating to the set-up of experiments influence the results.

The use of insect for cleaning the environment: ento-moremediation

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Entomoremediation (from the Greek *entomon* – insect and the Latin *remedium* – to clean or restore) is a fairly new subtype of bioremediation and can be defined as the use of specialized insects and their associated microorganisms to utilize, extract, se-quester and/or detoxify pollutants from soil, sediments and organic biomass. The first mention about entomoremediation was in theoretical work of Ewuim in 2013. The first article in which this word was used intentionally and which provides the results of experimental work was in 2018. There exist many publications on studies of heavy metal content in insects have been devoted to ecotoxicological contexts or to the safety of feed and food produced from them, which showed that insect are able to bioaccumulation of some elements. Entomoremediation comes out ahead and tries to put this phenomenon into practice. This approach is not very widespread while, similar to e.g. phytoremediation, it has unique characteristics that allow it to be distinguished from general bioremediation and make this distinction valuable. Distinguishing features of entomoremediation and results of recent published experiments will be discussed during the presentation. The research presented in the paper was partially financed by the Poland National Science Centre, as a result of the project no. 2019/35/D/NZ9/01835.

Modification in composition of black soldier fly puparium, prepupae and adults after *Lactobacillus* fermentation

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In a contest in which the interest in renewable raw materials is growing considerably, solid state fermentation is often applied to valorize organic wastes, thanks to the count-less benefits this process can bring to the unexploited biomasses, such as the microbial stabilization and the simultaneous production of antibiotics and bioactive compounds. *Hermetia illucens* (Black Soldier Fly, BSF) is a non-pathogenic insect that is often used in the bio-conversion processes of residual biomasses into feed ingredients.

In this work, for the first time, a solid-state fermentation was conducted with 2 lactic acid bacteria (LAB) strains, isolated from food, on insect prepupae and insect-derived wastes (pupal membranes and adult insects at the end-of-life cycle) produced during bioconversion processes. The present work was undertaken to study the difference in the molecular composition between fermented and unfermented insect-derived waste materials, focusing on lipid, protein, and chitin fraction.

The bulk evaluation of fat, protein, moisture, and ashes composition of insect-derived biomasses was carried out using standard procedures (AOAC, 2002). Fatty acid pro-files were determined by GC-MS, whereas total amino acid analysis, glucosamine monomer content (after chitin hydrolysis) and the formation of possible peptides or chi-tin oligomers was carried out by LC/ESI-MS methodologies. A metabolomics approach by 1H NMR was also applied to get further insight in both lipid and polar insect fractions.

The results showed that the lipid fraction is the most affected by the fermentation: the fermented biomass had a higher percentage of lipids and a more complex fatty acid profile as compared to unfermented mass, with a fatty acid composition shifting from a typical one from BSF to a more typical one for LAB. Also, the protein fraction changes upon fermentation, especially in the amino acid composition, while the production of peptides was very limited. On the contrary no chitin oligomers formation was observed after the fermentation process.

This study shows how fermentation can modify the molecular composition of insect-derived waste, exploiting the typical metabolic behavior of fermenting bacteria. Further studied are encouraged to better understand possible functional properties of insect fermented biomasses and related applications.

Anti-arthritic activity of black soldier fly (*Hermetia illucens*) larvae protein derivatives

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Black soldier fly larvae (BSF) derived proteins are gaining popularity as sustainable pet food ingredients. These ingredients are known to exhibit strong antioxidant potential mainly due to their ability to donate hydrogen atom and/or electrons to counterpoise reactive oxygen species (ROS). Some of the commercially available anti-arthritic medications also work using the same mechanism, giving indication that BSF proteins could also have a possible role in the prevention of arthritis. During this study, we evaluated the anti-arthritic potential of BSF proteins using following assays: (a). proteinase inhibition; (b). erythrocyte membrane stability; (c). modulation of ROS production by activated macrophages; (d). modulation of ROS production by monocytes; and (e). cellular toxicity. We also evaluated the glucosamine content of these ingredients. Chicken meal, a common ingredient in pet food formulations was used as an industrial benchmark. Results of this study demonstrate the strong anti-arthritic potential of BSF proteins. These ingredients are not only useful in preventing the development of arthritis but could also possibly help in its curation due to presence of glucosamine. Additionally, some of our results indicated that chicken meal could possibly facilitate the progression of arthritis.

The effect of chicken manure and pre-consumer waste on black soldier fly (*Hermetia illucens*) larval performance at industrial scale

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The black soldier fly, *Hermetia illucens L*. (Diptera: Stratiomyidae), is commonly used in bioconversion due to its versatility and efficiency in reducing various types of organic waste, including fruit and vegetable waste and animal manure. Most studies have looked and investigated only one type of waste at a time, but varying mixtures can lead to improved larval performance and bioconversion. This study investigated the effects of mixtures of chicken manure with pre-consumer waste from 5% to 40% with a tray with only pre-consumer waste used as a control. Freshly hatched neonates (450 mg), collected within three hours of hatching, were placed on 60 kg of each mixture. The results for this experiment will be more applicable to industry as they were conducted on a larger scale in an industrial setting. The larvae were held at 28 ± 0.5 ˚C and this was repeated for 10 different batches of each chicken manure and preconsumer waste mix. The performance of the larvae for different waste percentages were recorded and compared. Individual and total larval masses were similar between treatments. Development time was 22 days on average for the control trays and 25 days on average for treatments containing chicken manure. Survival was highest for control trays and lowest in trays containing 40% chicken manure. The highest waste reduction was achieved in trays that contained 10% chicken manure. Larger scale experiments and mixing low quality waste like poultry manure with preconsumer waste can be a viable option for waste reduction of and production of larval biomass from different available waste streams.

Extraction of chitosan from insect chitin

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Aim was the process development for a chemical and enzymatic extraction of chitin from insect raw material of locusts (*Locusta migratoria* and *Schistocerca gregaria*), in particular for the production of novel biogenic and degradable flocculants. Chitin is currently still extracted from marine biomass as crustaceans or zooplankton. This is economically and ecologically risky because of the use of large amounts of chemicals (hydrochloric acid, sodium hydroxide) and washing water. An efficient and environ-mentally friendly process for chitin extraction from insects shall therefore be developed within the project. It was also intended to produce these substances in pure forms for applications in medicine, health products, technology, and others. As an example, chitosan-based flocculants should be developed in this project for precipitation processes in wastewater technology in order to obtain biodegradable sludge.

Dried locusts were milled and de-oiled using n-hexane as solvent. The gained meal was further milled. For the de-mineralisation a fermentation step was used. The de-protein-ation could be achieved using NaOH and the de-acetylization also using NaOH but dif-ferent conditions. In order to develop applicable process steps the product yield, the chi-tin-content and the total nitrogen content were targeted. The de-acetylation of chitin to chitosan made it possible to convert insoluble or poorly soluble chitin into soluble chitosan and therefore the range of applications can be expanded significantly. A further chemical modification increased the solubility of chitosan whereat the amino and hydroxyl groups of the chitosan molecule reacted. IR and DSC investigations were carried out on all start-ing materials and products.

Development of a method to determine chitin in insect protein meal and insect farming by-products

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Insects are used as an alternative sustainable protein-rich ingredient in fish, pet, pig and poultry diets. Insect meals have a considerably good nutritional composition with regard to amino acids, minerals or fatty acids for feeding animals. However, the significant difference between insect meals and common protein sources is the content of chitin.

Chitin is an amino polysaccharide present in yeast cells, exoskeletons of insects and arthropod cuticles and is one of the most abundant biopolymers. In general, it is a homopolymeric chain of β-(1–4)-linked N-acetyl-D-glucosamine units, which has a linear fibre structure with a high amount of hydrogen bonds between the single chitin molecules. The polymer properties like solubility, bioavailability and reactivity are similar to cellulose. In the classical nutritional score analysis (Weender analysis) chitin is determined in the fibre fraction together with many other polymeric compounds. In addition, chitin also influences the determination of crude protein in the product. The nitrogen contained in chitin, which makes up approx. 13% of the chitin mass, is detected as protein in the analysis and therefore deludes the crude protein content in a higher range. Furthermore, β-glucans in particular have been identified as active immunostimulating agents. This all shows that there is a need for a method to determine the chitin in insect products separately.

Analysis methods described in literature consist of a total hydrolysis under hard conditions and a determination on the monomeric level with a colour reagent, which reacts with the nitrogen. These methods have some problems with complex matrices like insect meals. A closer examination of these methods shows that a total hydrolysis is not easy to handle, so mostly only a partial hydrolysis is achieved. Another critical aspect is the colour reagent itself, which not only reacts with the nitrogen in chitin but also with proteins, peptides or amino acids. So all in all, these methods are not able to determine the chitin content separately without an isolation of chitin in an insect meal. With this background, we developed a method to determine chitin in complex protein-rich matrices. This method enables a determination of chitin on a polymeric level without a preceding hydrolysis step. It allows a quantitative analysis of chitin and a correction of the crude protein content as well as an improved nutritional score rating.

Host microbiota profile, interactions, and vertical transmission across two generations of *Hermetia illucens*

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The black soldier fly (*Hermetia illucens*) has gained increased importance as a prime candidate for industrial production of alternative animal protein production and biowaste recycling. Optimisation of rearing management of this emerging industry requires a better understanding of how biotic factors, mainly host-microbiota interactions, affect the insect health and growth performance. This investigative study seeks to characterise microbiota associated with *H. illucens* across the entire life cycle (eggs, larvae, prepupae and adults) when reared in two substrates: i) plant-based (Housefly Gainesville diet) and ii) animal-based (poultry hatchery waste). We used a metataxonomic approach with 16S and 18S genetic markers amplicon sequencing, the latter made possible by the development of a blocking primer specific to the black soldier fly. This enables for the first time to produce a comprehensive profile of *H. illucens* microbiota encompassing a wide diversity of eukaryotic microorganisms, often neglected in microbial studies. The microbiota was sampled for two generations, from parents (X0) to all progeny's developmental stages until maturity (X1). OTU tables issued from amplicon sequencing are now being investigated to compare taxonomic and functional profiles, diversity and dynamics of populations and to reveal interactions networks within said profiles. We hypothesize the existence of a core microbiota that will be partly inherited from parents and remains stable throughout development, and we also expect the overall microbiota to be modulated by the feed substrate, developmental stage, and time. We further hypothesize that individuals not reared on the same substrate as their parent will display a greater divergence in core microbial composition and potential signs of dysbiosis in early stages. These preliminary results are expected to be completed in September. Our study will give insight into how *H. illucens* health and performance could be managed using probiotic supplements.

Influence of organic side stream nutritional composition on the performance of *Hermetia illucens* larvae

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The global population is growing and is expected to reach 9.7 billion by 2050. Along with this growth, the demand for natural resources is rising. Meanwhile, annual waste generation is expected to increase to 3.4 billion tons per year in 2050, of which 50% consists of organic waste. Both the depletion of natural resources and the increasing generation of waste are detrimental to our planet and society.

Larvae of the black soldier fly (*Hermetia illucens*) (BSFL) are voracious feeders that can grow on a wide variety of organic materials. When grown on organic residues, they are able to convert such low value materials into high value biomass composed of fats, proteins and chitin, which can be used as a sustainable resource for food, feed and technical applications.

In the light of the FACCE SURPLUS project UpWaste, 14 low-value organic residues were collected and chemically characterized. Approximately 6 months later, 8 of these side streams were sampled and chemically analyzed again in order to evaluate the stability of the stream characteristics in function of time. For all sampled streams a lab scale feeding trial with black soldier fly larvae was set up. Therefore, the streams were minimally pre-treated. The streams were fine-ground and the moisture content was adjusted to 30% dry matter. Survival rate, maximal larval weight, required time to reach maximal weight, bioconversion efficiency, waste reduction and larval behavior were examined.

Based on the chemical analysis of the side streams, caloric contents could be estimated. For some side streams, these contents were relatively constant in function of time, however other streams such as grain middlings had a 50% decrease in caloric content between different sampling dates. Also, the difference in caloric content was clearly visible in lab scale breeding trials. These differences empathize the importance of using multiple sampling dates.

Data of both chemical analysis and lab scale breeding trials confirmed the ability of BSFL to grow on a wide variety of organic materials. However, based on nutritional composition of the side streams significant differences were observed in larval performance.

Zootechnical performance improvement of black soldier fly (*Hermetia illucens*) by the addition of live yeast probiotics on the feed substrate

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Black soldier fly (*Hermetia illucens*) is a newly domesticated species in which insect producers still are in process of identifying its optimum nutritional requirements in the production process. Based on that, it is been found that it is possible to improve its zootechnical performance by providing it better feed conditions on the substrate.

The purpose of this research is to evaluate the zootechnical performance of black soldier fly (BSF) when adding live yeast probiotics (*Saccharomyces cerevisiae*) to its rearing substrate. The trial was run in ENTOBEL HOLDING PTE. LTD. Based in Vietnam. The feed substrate used was brewery by-products, that after adding Actisaf® Sc47 at 0.06% (fresh weight) was pre-fermented for 3 days followed by adding first instar BSF larvae. The zootechnical performance of BSF reared in the substrate treated with Actisaf® Sc47 was compared to the BSF zootechnical performance reared in the substrate that was only pre-fermented.

The output results demonstrated that Actisaf®Sc47 improves the BSF zootechnical performance. The Bioconversion rate measured as Dry larvae weight/(Diet on a dry weight basis before feeding – Diet on a dry weight basis after feeding, was improved by +22%, while the larval protein and lipid yield was increased by 5.6% and 17% respectively, versus the nontreated control.

Variation in the bacterial community composition of black soldier fly larvae (*Hermetia illucens*) from consecutive, industrial cycles

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As for many other insect species, the gut microbiome of black soldier fly larvae (*Hermetia illucens*, BSFL) is crucial for diverse aspects of insect physiology, affecting the health and survival. Nevertheless, conclusions on the microbiota of BSFL of the majority of the studies reported in literature are based on only one or a few laboratory rearing cycles. To obtain insight in the inter-cycle variation of the microbiota, the bacterial community compositions of BSFL and their feeding substrate were assessed. Samples at two different time points during six consecutive rearing cycles of three industrial companies were investigated by deep sequencing of partial 16S ribosomal RNA gene amplicons. The specific aims of this study were (i) to assess how the bacterial community compositions vary over cycles within a production facility when the same rearing practices are maintained, (ii) to obtain insight in whether and to which extent bacterial communities of larvae and substrates vary between different companies, and (iii) to assess the influence of the bacterial communities of larvae and substrates on each other's microbiota.

The results imply that the larvae and substrate within a company consisted of a particular set of bacteria at each time point and that no specific changes occurred over the cycles. Between the companies, a number of zero-radius operational taxonomic units (zOTUs, used to classify groups of species based on DNA sequences), which are also reported in BSFL in literature, were present in each facility. Further, the composition of the bacterial communities of the larvae of the second time point were more similar to each other than the communities of the larvae of the first time point. This may hint at a selection process in the mid gut. We conclude that some species are more likely to be found in BSFL than other species and that the microbiota of BSFL constitutes of a core microbiome. Nevertheless, there is variation in presence and abundances of species over locations and substrates, likely depending on abiotic and biotic factors.

Individual density and rearing scale influence temperature profiles during black soldier fly rearing

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The interest in larvae of the black soldier fly for the purpose of academic research or their industrial exploitation has experienced significant growth worldwide. So far, research questions are mostly being addressed at lab-scale, while industrial operations are primarily engaged in upscaling their production. In both cases, key features justifying the larvae's popularity such as their nutrient composition, conversion efficiency, and versatility in waste degradation depend on the configuration of rearing parameters.

However, the individual density, a parameter commonly provided as number of larvae per unit area, can blur the comparison of experiments and may inhibit reproducibility across different scales. Eventually, the total number of larvae derived from individual density × area of the rearing unit modulates their natural aggregation intensity during feeding. Despite equal densities, a higher total number of larvae at larger scales can therefore result in unpredicted temperature peaks, inducing stress events that range from flight reactions to inhibited growth and increased mortality.

Here, we report the effect of larval density (0,1.25, 2.5, 5 larvae cm^{-2}) on substrate temperature profiles during larval development on food wastes. To account for heat-input by microbial activity, we included a second treatment group where a conventional preservative was added to the substrate. In addition, temperature profiles throughout a large-scale trial at a density of 5 larvae cm^{-2} were monitored.

For the lab-scale experiment, peak temperatures increased by approximately 2 °C for each doubling of the larval density, with the highest density exceeding the ambient temperature by 6 °C. In comparison, at large scale the temperature peaked 16 °C above the ambient temperature. While the preservative delayed temperatures peaks, it also stalled larval development, thereby attributing importance to the substrate microbiota.

As excessive temperature peaks affect the rearing dynamics as a whole, care should be taken in describing all factors that could contribute to their occurrence. Therefore, standardizing rearing parameters not linked to the research question may help to increase comparability across experiments.

The super power of fungi: unraveling their metabolic effects on black soldier fly larvae

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While the metabolic interactions between microorganisms and insects are known from bacteria, an understanding of the metabolic interactions between eukaryotic microorganisms (e.g. fungi) and insects is lacking and very complex to study. The black soldier fly (BSF) (*Hermetia illucens L*.), is an omnivorous detritivorous insect that feed on decaying organic material and encounter diverse fungal community. Therefore, we predict that the BSF larvae (BSFL) will have metabolic interactions with the fungal community in its environment. These interactions can be due to consumption of the fungi and direct absorption of essential nutrients produced by the fungi or by digestion of indigestible nutrients by the fungi, thus providing available nutrients for insect consumption. In this study, we collected BSFL from household composts (natural populations) and identified the fungal community composition colonizing the BSFL gut using Sanger and NGS sequencing. In addition, we isolated specific fungi species and conducted feeding experiment to test their effect on the life history characteristics of the larvae. In order to identify the metabolic pathways that affects the fungi-BSFL interactions, we analyzed the metabolome of the larvae using HPLC. We found various fungi species that are common in the BSFL gut, for example, *Candida tropicalis, Kluyveromyces marxianus* and *Hanseniaspora pseudoguilliermondii*. The feeding experiment revealed increase in the body weight of the larvae fed with these fungi, compared to larvae fed with baker's yeast *Saccharomyces cerevisiae*. The metabolome analysis revealed several metabolic pathways that can affect the larval life history characteristics, for example amino-acid metabolism. The metabolome analysis provided predictions regarding the metabolic interactions between the BSFL and fungi that will be further studied in specific experiments. This research start to reveal the black box of metabolic process in which the fungi affect the BSF and provide knowledge with potential application, such as manipulating the fungal community composition in the BSF diet to improve the insect's rearing process and ability to digest different organic compounds.

Poster Presentations

Topic 1–5

Exploring the production and export potential of cricket powder in Costa Rica

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Costa Rica is a tropical country with perfect environmental conditions to raise crickets, thus a possible creation of a large-scale cricket powder production hub is seen as an economic opportunity for the country. The aim of the present study was to explore the potential of producing and exporting cricket powder in Costa Rica, understanding if it is feasible to comply with the product and production requirements of the international market. A qualitative research was performed through semi-structured interviews to technical experts, producers and local entrepreneurs, a visit to a cricket powder production plant in Thailand and a 1-day stakeholders' workshop in Costa Rica. Results show that Mexico, the United States and Europe are potential markets for Costa Rica. The main buyers are food and pet food producers with an average demand of 0,5–2 tonnes of cricket powder per month. The most important product requirements identified are the protein content, a gluten-free product and a neutral aroma. For the production, it is required to have an enclosed facility, with temperature and humidity control, a proper food safety and quality management system in place, with a standardized feed and water disposing system. It is concluded that the current ecosystem in Costa Rica does not allow to meet the market and process needs required for commercializing cricket powder. Challenges like the current low production capability (micro level), the lack of technical experts on mass-producing crickets (meso level), the lack of specific regulation on insect production (macro level) and the poor knowledge on edible insects from a society perspective (meta level) must still be overcome. However, it is expected that in 5 years Costa Rica will be able to produce and export a minimum of 2 tonnes of cricket powder per month. The main identified opportunities that would allow such a production are the existent local knowledge on rearing insects (micro level), existence of well-established academic, research, and export promotion support institutions (meso level), government SME support programs (macro level) and the local expertise on exporting butterflies (meta level). The creation of a producer's association, more research and training on edible insects and appropriate legislation are proposed as necessary strategies to develop an adequate ecosystem to produce cricket powder on an industrial scale.

Nutritive value of wheat bran in yellow mealworms (*Tenebrio molitor*): towards feed efficiency optimization

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Complete diets that can fulfil all larvae nutritional requirements are needed to optimize feed efficiency in mealworms. However, the nutritional value of common feed substrates (such as wheat bran) has seldom been studied. A procedure was proposed to determine the digestibility of raw materials in mealworms and used to determine the nutritional value of wheat bran. A simple diet with wheat bran only was used. A total of 4 polystyrene plastic trays (54 x 39 x 9 cm), each including 150 g of fertile imagos, were used for egg laying. Wheat bran containing eggs from the imagos, was weekly extracted (for 21 d; 3 egg extractions) and mechanically separated to obtain 12 trays with eggs. The larval growth period lasted 90 days. To determine the nutritive value of wheat bran, a digestibility trial was performed from 48 to 62 d post-hatch. Four trays corresponding to the first laying period were used. Diet was offered from day 48 until day 62 post-hatch, when all feed provided was ingested (zero feed refusal). Wheat bran offered and faeces excreted during these 14 d were weighed, sampled, grounded and stored until further analysis. The wheat bran evaluated had the following composition: 171 g crude protein (CP), 123 g starch, 490 neutral detergent fibre (NDF), 142 g acid detergent fibre (ADF), 30 g acid detergent lignin (ADL) and 19.1 MJ gross energy (GE) per Kg of dry matter (DM). The apparent faecal digestibility coefficients were 39.0, 39.9, 15.9, 6.2 and 39.9% for DM, CP, NDF, ADF and GE, respectively. Values obtained for the CP and GE digestibility were low, probably influenced by the high fibrous content in wheat bran. Thus, the nutritional value of the wheat bran was 7.61 MJ of digestible energy and 68.4 g of digestible protein per Kg of DM. Our results showed that a simple diet based only wheat bran does not fulfil all nutrient requirements of mealworms, probably limiting their growth and nutrient utilization. Nutritional assessment of the raw materials should be a priority to design complete diets that meet mealworms requirements to optimize larvae production and the economic return.

Growth patterns in small-scale farmed edible crickets in Germany

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Although crickets (Gryllidae) have a long tradition of consumption, farming them in Europe seems less frequent than e.g. mealworms or BSF. One of the goals of the project "IFNext" was to develop sustainable farming systems for crickets. In Germany, a modular approach with medium-sized boxes, a special water supply and feeding a combination of chicken concentrate and vegetable cut-offs was created ("Krabbelkist"). The objective of this study was to describe the growth pattern of the reared crickets (*Gryllus bimaculatus* [n = 17 cycles], *G. assimilis/locorojo* [n = 9] and a combination of both species [n = 14]) to determine the ideal moment of harvest.

The life cycle was monitored by weekly weighing. For practicability, box and egg cartons weights were determined previously, while the complete box was weighed weekly, and after the cycle completed, animal weight was determined mathematically. As absolute weights varied strongly since they depended on the total amount of animals, values were expressed in percentages in relation to the tenth week. Each growth curve was analysed and represented by a mathematical function.

It was seen that according to the combination of the functions' signs (positive or negative), three different growth patterns occurred which differed significantly ($p < 0.05$) from each other in terms of yield. Interestingly, these patterns occurred in all three groups and sometimes even simultaneously, so that the feeding cannot be responsible for these patterns. Instead it is presumed that the cricket strains may be responsible as the colonies were refreshed periodically with animals from other breeders.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of der Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

Small-scale food cricket production in Thailand, Cambodia, and Germany using local feed sources

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Insect production can be achieved on many levels. "IFNext" is a project focusing on small-scale, sustainable insect farming to provide local farmers in Cambodia and Thailand with a technique to rear and produce, among others, crickets (*Gryllus bimaculatus, G. assimilis/locorojo,* and *Gymnogryllus vietnamensis*) and develop novel food products from them, to improve farmers' nutrition and income. In Germany, a small-scale rearing system was developed for crickets without the involvement of farmers.

In Asia, larger containers of e.g. 2.5 x 1.2 x 0.6 m (on a construction adding 0.3 m to the height) are used. Egg cartons fill the entire pen or only part of it; the cartons sometimes rest on a bamboo structure to ensure airflow. Humidity is maintained by e.g. banana leaves or rice straw, and water is administered in a tube system, which prevents the crickets from drowning. Feed comprises either a cricket-specific concentrate (Thailand) or chicken feed with local resp. seasonal plants (Cambodia). These plants are not (longer) used for human consumption, may be invasive or a sub-product from ordinary agriculture, e.g. from cassava growth. Many of them are rich in protein. Escape is prevented by taping the upper rim of the pen and a plastic mesh.

In Germany, the "Krabbelkist" consists of a medium-sized plastic box containing nine egg cartons, dishes for food and a modified plastic bottle for watering. Feed comprises chicken concentrate and vegetable off-cuts from a local supermarket, keeping costs low. German green feed contained less protein than the Asian one.

These systems are able to produce 6–14 kg fresh crickets/m³/10 weeks.

The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of der Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE).

Black soldier fly (BSF) production using the organic fraction of municipal solid waste

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The organic fraction of municipal solid waste (OFMSW) contains large quantities of organic matter (more than 85% dry matter) of high biological value, suggesting this fraction being recovered to produce high added value products. The SMARTFEED Project (founded by CARIPLO Foundation) aims to investigate the possibility of producing a healthy feed for laying hens through the integration of *Hermetia illucens* larvae, reared on the OFMSW, and microalgae grown on the liquid fraction of the digestate. The OFSMW collected in different season and subjected to different treatments, i.e. untreated solid waste texture and pulp slurry texture, was evaluated for its composition and performance as larval growing substrate and biogas production. The fiber content in the pre-treated pulp material ranged from 299 and 368 g kg^{-1} and was slightly lower than that of the untreated OFMSW, which ranged from 309 to 438 g kg^{-1}, likely due to hemicellulose removal. Larvae successfully grew on both treated and untreated OFMSW. No statistical differences were observed on the larval survival, which was always over 96%, and on the other measured indexes: substrate reduction, efficiency of the conversion of the ingested food and the larval growth rate. Similar biogas productions were observed between the two textures and there was no within-sample seasonal effect. A comparative material balance and process yields taking in consideration the amount of residual OF-MSW larval waste along the anaerobic digestion is presented. We sought to develop a Smart FEED production model that can be facilely integrated within the entire biorefinery where waste and residual biomass valorization are all essential unit operations of bio-conversion.

Carrot supplement enhanced the levels of lipophilic vitamins in Jamaican field crickets – preliminary results

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The overall composition of insects is known to be affected by multiple factors, such as species, developmental stage, rearing conditions, or composition of the provided feed. The insects are able to accumulate some specific substances such as vitamins or minerals in their bodies. Manipulative enrichment of the insect biomass by such nutrients may then contribute to better meet requirements of the final consumers.

Therefore, our research aimed to reveal whether the inclusion of carrot to the diet of Jamaican field crickets (*Gryllus assimilis*) may have an effect onto their life performance and nutritional value. In total, four experimental groups (where carrot was provided 3, 7, 14 and 60 days) and one control without carrot supplementation were tested in triplicates. All the groups were reared under the same conditions in the insectarium at the Czech University of Life Sciences Prague.

The one-way ANOVA and Scheffe's post-hoc tests revealed no effect of carrot addition into the diet on individual weight, feed conversion as well as on contents of dry matter, ash, fat, protein, tocopherols, and lutein. On the other hand, statistically significant differences ($p < 0.05$) were found in case of carotenes. Although significantly highest yield of carotenes was found in the crickets fed by carrot for all the development, high levels of carotenes were also detected in the crickets supplemented by carrot for 14 days prior to harvest only. Moreover, even the crickets fed by carrot only three days contained significantly higher levels of carotenes than in control group.

Strategies and suggestions for optimizing *Hermetia illucens* rearing

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As the concept of insects as food and feed is getting more and more popular, the industrial insect production is booming. However, some challenges need to be addressed to optimize production. The black soldier fly, *Hermetia illucens* (BSF; *Diptera: Stratiomyidae*), represents one of the insects valued for its high quality protein, fat, and its ability to thrive on a variety of organic substances. Plant-based side streams have the potential to be used as feed for BSF even under strict rearing regulations. Feeding trials followed by chemical composition analyzes help in understanding the nutritional requirements of the larvae. The dependence of insect protein-producing industries on only a few by-products will change the scenario to create higher demand for these by-products. Therefore, combining several side streams could be a way to prepare a sustainable and economical diet.

Besides larval dietary requirements, other factors also play a critical role in the development and maintenance of a BSF breed. When larvae are kept at a particularly high density, the temperatures of the substrate rise to peaks of up to 50 °C, resulting in morphological problems during ecdysis. We observed an increase in strongly helically deformed larvae at high stocking densities. Moreover, on an industrial scale, there is a risk that putative entomopathogenic microbes lead to monetary losses. Although the extent to which certain bacteria and fungi affect rearing success and yield is unclear, abundant microbes such as *Pseudomonas aeruginosa* seem to slow down the growth of the larvae. Based on this, the susceptibility of BSF larvae to entomopathogenic bacteria and fungi is currently investigated.

Finally, the mating of BSF depends on several factors. Due to seasonal fluctuations in sunlight, the identification of light sources with an adequate wavelength spectrum and energy efficiency acts as an important factor. The experimental results show that different LED light color treatments affected the oviposition peak. To this day, the targeted deposition of eggs also represents a bottleneck in BSF rearing. Here, a SPME-GC-MS based methodology was used to examine volatile substances that might influence the site of oviposition.

Influence of feed on nutritional quality *Gryllus assimilis*

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The growth of the world's population will require the production of big amounts of food. However, due to the limited area of pastures, it will be very difficult to meet this need with conventional food sources. This can lead to food shortages, especially of animal origin. It will be necessary to look for new sources of animal protein, such as insects, which have a high nutritional value. Since 2003, the Food and Agriculture Organization of the United Nations (FAO) has recognized the potential use of edible insects for food and feed and has supported a line of topics related to edible insects.

The nutritional value depends on the breeding conditions, the insect stage and technological modifications. An important factor that also affects the nutritional value is the type of food. The aim of this work was to assess the effect of the addition of rapeseed cakes (70% was added to the classic feed mixture for broilers, which has already proven itself in experiments with edible insects at the Czech University of Life Sciences) on the nutritional value of *Gryllus assimilis*. Rapeseed cakes are a by-product of hot pressing of rapeseed. It contains a high proportion of nitrogenous substances and a high proportion of fat, which ensures high nutritional quality in terms of protein and energy feed. At the same, it is a waste product which, in addition to animal feed, is used in biogas plants. In the Czech Republic, it is a relatively abundant product. The second group of crickets was fed a conventional soybean broiler feed.

It was found that crickets were able to thrive on both types of food. The nutritional values of both groups showed very similar values. Although there was an increase in fat content at the expense of protein, the resulting fatty acid profile in crickets feed rappesed cakes showed a lower proportion of saturated fatty acids and an improvement in the ratio of omega-3 to omega-6 fatty acids. In conclusion, rapeseed cakes can be recommended as a possible alternative to soy feed for crickets.

ValuSect: Valuable inSects

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The global population is growing and expected to reach 9 billion by 2050, while resources are decreasing. The NWE Area is a very densely populated area characterized by intensive agriculture, so it is necessary to find sustainable alternatives for food and feed resources. The Interreg NWE ValuSect consortium has 11 full (Thomas More Kempen vzw, Inagro vzw, Aberystwyth University, Zürcher Fachhochschule, Stichting Fontys, BB projects, Teagasc the Agriculture and Food Development Authority in Ire-land, New Generation Nutrition Pro-Active, AliénorEU, BIC Innovation Limited and Deutsche Gesellschaft für Züchtungskunde) and 9 associated partners (Flanders Food, BIIF, Greenport Westholland, Eurasanté, Pole Valorial, Food Processing, the Welsh Government, CCPA Groupe and The Belgian Feed Association) from 7 countries in the NWE area. ValuSect enhances the innovation performance of enterprises throughout NWE regions. The focus will be the development of a sustainable transna-tional Accelerator program including all partners with expertise on insects, food and feed production, innovation and commercialisation to support and cooperate with enterprises. Four insect species (*Tenebrio molitor, Acheta domesticus, Locusta migratoria* and *Hermetia illucens*) are considered in this project. The knowledge will be transferred by pilot demonstration, real life testing in close collaboration with end consumers and via open/thematic calls for cases (vouchers for SMEs). During the duration of the project 60 cases from companies/SMEs will be selected and performed. The next call for cases will be autumn 2021 and the knowledge platform is under construction. The accelerator program will be sustained after the project. This will increase the innovation levels of enterprises in the food and feed industry in the NWE Area and will accelerate the creation of a consumer market for insect food applications in the long term.

Substrate fermentation evaluated as a strategy to improve the ω-3 content of black soldier fly larvae (*Hermetia illucens*)

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Due to their beneficial content of proteins and lipids, black soldier fly larvae (BSFL) are a good candidate as a nutrient source for feed. However, their content of polyunsaturated fatty acids (PUFAs) is currently low, in particular regarding their use in aquafeed. It has been shown that the PUFAs of BSFL could significantly be increased by enrichment of their rearing medium with ω-3 fatty acids. Here, the potential of microbial fermentation to enrich nutritionally low-value substrates with PUFAs and increase the ω-3 content of larvae reared on these substrates was investigated. Spores of candidate species *Pythium irregulare* and *Mortierella isabellina* were used to inoculate wheat bran- and dried distiller's grains with solubles (DDGS)-based substrates. Optimal growth conditions for P. irregulare were reached with a dry matter content of 20%, a 70:30 ratio of wheat bran to DDGS, 10.5 days of incubation and inoculation of 4,4 log spores/g. For *M. isabellina* optimal conditions were reached at 50% DM, 100% wheat bran, 14 days incubation and inoculation of 4,4 log spores/g. Fermentation of substrates with *P. irregulare* and *M. isabellina* led to an increase in fat content of 53,7% and 68%, respectively. However, no significant increases in the ω-3 content of the substrates were observed. After successful fermentation, substrates inoculated with either *P. irregulare* or *M. isabellina* were used as feed for BSFL. For the experiment with *P. irregulare*, two control groups were used as feed, where one control group was sterilized before feeding, while the other control group was freshly prepared and non-sterile. Five hundred 6–8 days old larvae were used per treatment. All experiments were done in triplicate. Larvae were placed in a container on 100 g fermented- or non-fermented (control) substrates. Larvae were fed and weighed during an 8 day period, after which larval weight, larval survival and frass weight was recorded. Larvae reared on either fermented substrate showed no growth increase and low survival rates com-pared to the control groups, suggesting complications with the consumption of the fermented substrates.

Systematic studies on the antioxidant capacity and volatile compound profile of yellow mealworm larvae (*Tenebrio molitor L.*) under different drying regimes

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The yellow mealworm (*Tenebrio molitor L., Coleoptera: Tenebrionidae*) is an edible insect and due to its ubiquitous occurrence and the frequency of consumption, a promising candidate for the cultivation and production on an industrial scale. Moreover, it is the first insect to be evaluated by EFSA following the novel food application. Drying is an important preservation step in industrial insect mass production and processing. Currently, information on the stability of nutritionally relevant, functional and sensory components of mealworms during drying is still scarce. The focus of the present study was to analyze the antioxidant capacity and volatile profile of mealworm larvae dried in various regimes (freeze-dried, microwave-dried, infrared-dried, oven-dried and high frequency-dried). To summarize 1) Fresh mealworm larvae do have slow and fast acting lipophilic and hydrophilic antioxidants. 2) The drying process was decisive with regard to the extraction efficiency of these antioxidants. 3) The highest antioxidant capacities were found in the microwave or oven-dried larvae, while the extracts obtained from the freeze-dried larvae had the lowest values. 4) By analyzing the volatile components, the present study provides insights into the extent to which Maillard reaction and lipid oxidation take place in the course of drying, and the results underpin the impact of the drying procedure.

Deepening the knowledge of process-induced changes of mealworm quality will contribute to improving *Tenebrio molitor L*. processing technologies, a basic prerequisite for utilizing mealworms as novel food or animal feed in the future.

Alternative processes for the production of chitosan from house crickets (*Acheta domesticus*)

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Edible insects have been identified as a promising source of chitin. Chitin and its derivative chitosan have a wide range of applications in various sectors, including the food industry. Chitin is usually isolated with sequential alkaline and acidic treatments, which generate chemical waste and affect the properties of the produced materials. In the present study, alternative methods were compared to the chemical treatment for chitin isolation from house crickets (*Acheta domesticus*). Chemical demineralization was compared to fermentation with *Lactococcus lactis*, treatment with citric acid and microwave treatment, leading to a degree of demineralization of 91.06±0.34, 97.33±0.79, 70.51±3.5 and 85.80±1.29%, respectively. Fermentation with *Bacillus subtilis*, a deep eutectic solvent and enzymatic digestion with papain and bromelain were tested for chitin isolation, but the chitin content of the generated materials was less than half when compared to the alkaline deproteinization. Further, chitosan was produced by deacetylation of the chitinous material obtained from two processes at a large scale: the chemical treatment and an alternative process by combining *L. lactis* fermentation with bromelain deproeinization. The chemical and alternative processes resulted in similar chitosan content (81.92 and 88%), antioxidant activity (59 and 49%) and degree of deacetylation (66.57 and 62.92%) respectively. The chitosan products were characterized with Fourier-transform infrared spectroscopy (FTIR), Thermogravimetric analysis (TGA) and Scanning electron microscopy (SEM), which indicated comparable results. Therefore, the alternative process presented a good potential to replace chemical process of chitin isolation for industrial applications.

Protein hydrolysates from edible insects: agricultural applications

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Insects are a good source of proteins so they are considered a sustainable and environmental friendly alternative for both animal feed and human diet, and furthermore, their harvesting/rearing requires a low-tech and low capital investment. For those reasons, protein obtained by insect breeding open interesting possibilities, i.e., application in agriculture as biostimulants based on protein hydrolysates. The aim of this work is to study different methodologies to obtain the best profile of insect protein hydrolysates. Briefly, *Zophoba morio, Tenebrio molitor* and *Hermetia illucens* larvae were selected for rearing. Then, the protein and amino acids content were evaluated and the amino acids profile was determined by means of enzymatic and acidic hydrolysis reaction followed by HPLC analysis. According to the results, *H. illucens* larvae reveals a higher total protein content (51.6%), while the other species only contain 42–47%. The highest percentage of soluble protein is found in *T. molitor* larvae (6.5%), followed by *H. illucens* (5.1%), being *Z. morio* larvae the insect with the lowest soluble protein content (3.3%). All the species showed a very low amino acids value: ~0.014%. In order to obtain protein hydrolysates from insect larvae, three different hydrolysis methodologies were evaluated: two enzymatic and one acidic. The results revealed that all hydrolysis methods did not achieve a complete hydrolysis due to the low amino acid content obtained. However, the residual value of soluble protein that remained unhydrolyzed suggest that the soluble protein fraction was efficiently hydrolyzed. The data obtained show that acid hydrolysis give the best results with higher amino acids content (1.0%), while only 0.2–0.7% are obtained with the enzymatic processes. The amino acid composition of the protein hydrolysate mainly consists of lysine (8.0%), leucine (7.7%), and valine (7.2%). On the other hand, it should be noted that in all cases the value of amino acids is increased compared to the non-hydrolysed larvae, suggesting that the hydrolysis processes are effective methodologies in order to hydrolyze the protein soluble fraction, which suppose a small percentage of total protein content. In conclusion, the above results showed that hydrolysis methods open future research for obtaining protein hydrolysates with applications in agriculture.

Description and characterization of the fluorescence excitation emission matrix of powders from selected edible Orthoptera species: A chemometric approach

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Fluorescence spectroscopy coupled with chemometric tools is a powerful analytical method largely used for rapid food quality and safety evaluations. However, its potential has not yet been explored in the area of edible insect processing. In the present study, excitation emission matrix (EEM) of 15 insect powders produced by milling insects belonging to 5 Orthoptera species (*Acheta domesticus, Gryllus assimilis, Gryllus bimaculatus, Locusta migratoria, Schistocerca gregaria*) from 3 different origins was recorded and investigated. Parallel factorial (PARAFAC) analysis performed on the overall averaged dataset was validated for five components, highlighting the presence of five different fluorescence peaks preserved among the several insect species. Slight differences on the fluorescence signal intensity among different samples were attributed to different species and origins. Identification of PARAFAC components was performed by evaluating the adequacy of the original PARAFAC 5-components model after adding the spectra of one pure standard compound. Significant improvements were observed when EEM of albumin, tryptophan, tyrosine, tocopherol, collagen, pyridoxine, pterins and NADH were alternatively added to the initial dataset. Comparison between component emission loadings and spectra of the aforementioned pure standards allowed to attribute component one (centred at ex/em 295/300 nm with shoulder at 333 nm) to mixture of tryptophan, tyrosine and tocopherol, component two (centred at 285/333 nm) to proteins (mixtures of tyrosine and tryptophan) and component three (centred at 345/390 nm with shoulder at 420 nm) to mixtures of collagen, pyridoxine and pterins. In conclusion, this study shows that fluorescence spectroscopy could present a potential tool for investigating the composition and quality of insect-based ingredients and foods.

The influence of the Maillard reaction on insect products and their nutritional score

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The non-enzymatic browning, the so-called Maillard reaction (MR) is since the last decades a well-known reaction in food technology. In general, the MR is a reaction between any reducing saccharides and organic nitrogen compounds like proteins, peptides, amino acids or other amines. The reaction is greatly accelerated by temperature and can be induced under both wet and dry states. The MR has an effect on the nutritional score of products and is also often used to influence various product aspects, such as colour, taste, smell and digestibility. Furthermore, the MR induces the formation and enrichment of process contaminates which are potentially genotoxic and hazardous like acrylamid. Regarding these contaminates the European Food Safety Authority (EFSA) has published a guideline in 2012 including the Margin of Exposure approach. In addition, the EFSA published the COMMISSION REGULATION (EU) 2017/2158 with establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food.

Taking this into account, we started a critical overview of the processing of insects in food and feed. In processing, drying steps are often used to create a hygienic manageable product, however there is mostly insufficient knowledge about the effects between the nutritional score and the process contaminates. We have observed a significant change in the amino acid composition in relation to the drying technology and the process parameters. In addition, we have seen a significant improvement of the solubility of proteins, which is a fairly good indication for the bioavailability in the digestive tract.

Palatability improvement potential of black soldier fly larvae protein hydrolysate in pacific white shrimp diets

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With an increase in consciousness for overfishing, fish feed producers are shifting towards plant-based protein sources. According to the literature, plant-based protein diets for shrimp are associated with low attractability and palatability. Commercially, cephalopods (e.g., squid) and crustaceans (e.g., krill) derived ingredients are used to improve the palatability of shrimp diets. However, production of these ingredients are also associated with several environmental issues including overfishing and biodiversity loss. Insect derived ingredients are not only sustainable and could also help in improving the health of consuming animals. Ingredients such as insect protein hydrolysate could also have a potential role in enhancing palatability of shrimp diets.

In this study, we evaluated the potential of black soldier fly (BSF) larvae protein hydrolysate to replace commercial squid meal and krill oil for the purpose of palatability enhancement. During this study, we investigated the following parameters: a) crude composition of four different diets; b) time required by first shrimp to begin feeding (time to strike); and c) palatability improvement.

Results of this study indicates significant palatability improvement with inclusion of BSF larvae protein hydrolysate. Improvement of palatability could be attributed to high free amino acid content in comparision to commercially used feed ingredients.

Survival of black soldier fly larvae (*Hermetia illucens*) in water at different temperatures: potential for storage and transport

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As a result of the importance of black soldier fly larvae (BSFL) in the insect sector, demand and production of this species are increasing quickly. Consequently, a larger scale production of BSFL involves an increased importance of transport and storage of the insects as part of the logistic chain. The management of this logistic chain within or between companies producing and/or processing BSFL is essential in order to prevent economical losses and optimize the insect sector. Research regarding methods for stable transport and storage of insects is scarce and therefore necessary.

Water can be an promising transport or storage medium for living insects. It not only provides the possibility of combining washing and storage/transport, but can also contribute to fast and easy cooling of the insects and can allow the insects to be “pumped” through or between facilities. A high survival is of course crucial when applying a storage technique. Hence, in this study, the survival of BSFL in water of different temperatures is currently being investigated as a first step in assessing the potential of water in insect storage or transport. Samples of living, freshly harvested BSFL are submerged in preconditioned water (3:1 ratio insects:water (m/m)) at three different temperatures (ambient, 15 °C and 4 °C) and stored for five days at their respective temperatures. With intervals of 12 to 24 h, samples are being evaluated for the survival of the larvae. Additionally, the pH of the water during the storage period is monitored in order to investigate the impact of the residing larvae on the pH of the water over time. In a next study, also the impact of the microbiological quality of the water and the insects as well as excretion of frass during storage on the survival rate can be included. Based on the results from this and following studies, guidelines for storage or transport in water can be developed for the insect industry.

In what context do you want to eat edible insects?

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In Europe, edible insects are unusual in food habits. Food neophobia and disgust are considered as major barriers to western entomophagy development. Strategies to improve the consumer acceptance are discussed in the literature but are mainly related to product and individual factors. However, other factors are involved and need attention as contextual factors. Defining the most appropriate context for insect consumption is a milestone in the insect-based food development and the context encompasses a set of parameters: “for which audience”, “at which time”, “in which place”, “in which social environment”, etc.

A preliminary study was conducted to record the first trends related to this topic by focusing on the timing of insect consumption (i.e. breakfast, lunch, dinner and between meals) and the style of insect preparation (i.e. main dish, trimming and snack) as well as challenging the questionnaire for further large scale studies. The survey was carried out at Gembloux Agro-Bio Tech – University of Liège (Gembloux, Belgium) with 475 respondents (mean age: 27.62 ± 11.45 years old).

Only one respondent did not know that insect could be used as food and many respondents would be willing to eat insects. Insect consumption was already experimented by most respondents but in an uncommon way (i.e. yearly or less frequent) and mainly limited to tasting during special events (e.g., conference). Concerning the context of insect consumption, the breakfast was considered as the less appropriate time to consume insects. Presenting edible insects as snacks seemed the most appropriate followed by insects as trimming.

Modular environmental and economic assessment applied to the production of *Hermetia illucens*

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Problem: The inclusion of insect protein into the food system has been suggested as a promising solution to ensure future food security and mitigate adverse environmental impacts related to food production. The market volume for edible insects in Europe is still small. Producers require a decision-support system to ensure a sustainable upscaling of the sector. To this end, insect production's environmental and economic impacts were analyzed to identify the most eco-efficient production scenario.

Methods: A modular eco-efficiency assessment approach was developed and applied to the production of dried *H. illucens* larvae. A hypothetical industrial-scale insect production system was disaggregated into 29 modules which can be recombined to represent 4608 distinct production scenarios, characterized by different feeds, energy efficiencies, and processing technologies. Environmental life cycle assessments and cost assessments were carried out in parallel for each module, and eco-efficiency assessment was used to assess these two sustainability dimensions jointly. The influence of the insect feed on the performance and thus impact of the production system was investigated by employing feed-specific scaling factors to the aggregation of module results to production scenario.

Result: The most eco-efficient production scenarios include highly energy-efficient rearing facilities and use blanching and microwave drying for processing. The insect feed is the largest contributor to the environmental impact and cost, but from an eco-efficiency standpoint, the choice of feed is, to a great extent, not crucial. Waste-type feeds (manure; fruit and vegetable waste) have low environmental impacts and costs, but the production systems based on these feeds are less efficient. The low impact of the feed is offset by the higher impact of the rearing and processing stages. Conversely, systems based on higher-quality feeds (by-products like wheat middlings or distiller grains) require fewer resources, but the feeds' impact and cost are higher. Only highly processed feeds, such as compound chicken feed, should be avoided for insect rearing.

Effects of sewage sludge recyclate supplementation of substrate on cadmium, lead and iron contents in BSF larvae

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Black soldier fly larvae (BSFL) can produce protein from low-quality nutrient sources and may help to reintegrate minerals in the nutrient cycle. Current sewage sludge processing is linked with the recycling of minerals, e.g., by pyrolysis (PY) or the Ash2Phos process (As2). When using these products as supplements in animal nutrition, the enrichment of heavy metals is thought to be a problem. We added 4% of these two recyclate types as supplements to a modified Gainesville diet (GD) using 8000 larvae in a 40 x 60 cm crate with 10 kg of feed (wet weight) and chicken starter feed (CF) as control substrate (4 diets with 6 replicates each). We analysed the incorporation of the heavy metals cadmium (Cd) and lead (Pb) but also the trace element iron (Fe) in BSFL. The PY recyclate had a high Fe content (141.1 g/kg dry matter (DM)) compared to the As2 product (430 mg/kg DM) while Pb (87.2 vs. 1.9 mg/kg DM) and Cd (0.24 vs. 0.02 mg/kg DM) were higher in PY. Mineral enrichment per kg DM was analysed using the GLM procedure of SAS. The Pb content in the BSFL increased in the order of PY > As2 > GD = CF (1.2 > 0.5 > 0.2 = 0.2 mg/kg DM). Pb was higher in PY than in As2 larvae ($P<0.001$) and both were higher in Pb than in GD and CF BSFL ($P<0.001$). The content of Cd was similar in PY (0.77 mg/kg) and As2 BSFL (0.74 mg/kg). In PY BSFL Cd was higher ($P<0.01$) and As2 tended ($P<0.1$) to be higher than in GD (0.62 mg/kg). Cd was lower in CF larvae (0.18 mg/kg) compared to GD, PY and As2 ($P<0.001$). Most Fe was observed in PY BSFL (556.6 mg/kg), which did not differ from As2 BSFL (339.2 mg/kg) but Fe was higher than in the GD (136 mg/kg) ($P<0.01$) and the CF BSFL (263.7 mg/kg) ($P<0.05$). In conclusion, the composition of the PY recyclate, rich in carbon, may pre-vent the enrichment of Pb in the larvae but had no effect on Cd, which is known for bioaccumulation in BSFL. Highest Fe in larvae was observed with the PY supplement, but the Fe uptake from PY seems to be strictly controlled. According to the EU legislation (2002/32/EC), concentrations of both heavy metals PB and Cd are below the threshold for complete feed (Pb 5 mg/kg DM; Cd 2 mg/kg DM). These data help to assess the potential use of sewage sludge recyclates as a source for minerals for BSFL.

A molecular survey of bacterial species in the guts of black soldier fly larvae (*Hermetia illucens*) reared on two urban organic waste streams in Kenya

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Globally, the expansion of livestock and fisheries production is severely constrained due to the increasing costs and ecological footprint of feed constituents. The utilization of black soldier fly (BSF) as an alternative protein ingredient to fishmeal and soybean in animal feed has been widely documented. The black soldier fly larvae (BSFL) used are known to voraciously feed and grow in contaminated organic wastes. Thus, several concerns about their safety for inclusion into animal feed remain largely unaddressed.

This study evaluated both culture-dependent sequence-based and 16S rDNA amplification analysis to isolate and identify bacterial species associated with BSFL fed on chicken manure (CM) and kitchen waste (KW). The bacteria species from the CM and KW were also isolated and investigated. Results from the culture-dependent isolation revealed that *Providencia* spp. was the most dominant bacterial species detected from the guts of BSFL reared on CM and KW. *Morganella* spp. and *Brevibacterium* spp. were detected in CM, while *Staphylococcus* spp. and *Bordetella* spp. were specific to KW. However, metagenomic studies showed that *Providencia* and *Bordetella* were the dominant genera observed in BSFL gut and processed waste substrates. The diversity of bacterial genera recorded from the fresh rearing substrates was significantly higher compared to the diversity observed in the gut of the BSFL and BSF frass.

These findings demonstrate that the presence and abundance of microbiota in BSFL and their associated waste vary considerably. However, the presence of clinically pathogenic strains of bacteria in the gut of BSFL fed both substrates highlight the biosafety risk of potential vertical transmission that might occur, if appropriate pre-and-postharvest measures are not enforced.

Impact of heat treatment on the microbiological composition and safety of frass of black soldier fly larvae (*Hermetia illucens*)

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Despite the high substrate conversion rate of insects, the production of by-products such as insect faeces, unconsumed substrate and exuviae is inevitable. These insect by-products, also called frass, have been gaining more and more interest as potential organic soil fertilizer. An important point of attention in this respect is the possible presence of food pathogens, since only safe residues should be used to spread on agricultural land. Hence, there may be a need for treatments that reduce harmful organisms, such as a heat treatment. In this context, European legislative regulations are set (Regulation (EU) No. 142/2011) to align treatment procedures based on the standards for animal manure, i.e. a heat treatment of 70 °C for one hour, to meet specific micro-biological criteria for *Clostridium perfringens*, *Salmonella* and Enterobacteriaceae.

In this study, the impact of a heat treatment on the microbiological quality of black soldier fly frass was examined. Several samples were inoculated with either *C. perfringens* or *Salmonella* spp. at a level of approximately 5 log cfu/g and heat treated at 70 °C for one hour. The time-temperature profile of the coldest point in the sample container was monitored to assure the heat treatment was applied correctly. Microbial counts were determined before and after the heat treatment. After the heat treatment, results showed *Salmonella* spp. to be absent in 25 g of frass and Enterobacteriaceae to be below the detection limit of 1 log cfu/g, both meeting the legislative criteria. In addition, the reducing effect on the total viable count and aerobic endospores was studied, showing no reduction for aerobic endospores and only a slight reduction for the total viable count. Experiments with inoculation of *C. perfringens* (which are currently being performed) will reveal if the heat treatment of 70 °C for one hour will also be sufficient to meet the third microbiological criterium set in legislation, i.e. absence of *C. perfringens* in 1 g of frass.

Effect of black soldier fly larvae on horse fecal emission of methane

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The equine sector has increased globally during the last decades and currently about 3 million hobby and sports horses reside in the EU member states. This increase is associated with large amount of horse manure. Decomposition of manure is associated with emission of greenhouse gases, primarily in the form of carbon dioxide, methane (CH_4) and nitrous oxide. Methane is a strong greenhouse gas with important roles in controlling concentrations of hydroxyl radical in the troposphere and of ozone in the stratosphere. Black soldier fly larvae (BSFL) may have the potential to reduce CH_4 emission because they can recycle large amounts of carbon into insect proteins and oils. This study evaluated whether presence of BSFL in horse feces modify direct emission of CH_4. To do so, feces sampled from one particular horse were incubated in a custom-built multiplexed automated flux system that allows automated hourly measurements of CH_4 in three opened dynamic chambers in turn. One chamber contained 500 g of feces and the two others contained 500 g of feces with 100 BSFL of mixed ages. Before putting them in the chambers, the larvae initially provided by INAGRO® were bred on feces. Hourly CH_4 emissions were measured with a Guardian® gas analyzer during 17 consecutive days. The experiment was replicated twice. The mean amount of CH_4 measured in the chamber containing only feces was not significantly different from that measured in the ambient air. Inversely, the mean quantity measured in chambers with BSFL was higher, between 13 and 27 ppm by volume, than the quantity measured in the chamber containing only feces. These results suggest total direct CH_4 emissions from horse feces are affected by the presence of BSFL. In a review of CH_4 production in pig manure, it was shown that lack of oxygen, high temperature, high level of degradable organic matter, high moisture content, low redox potential, neutral pH and C/N ratio between 15 and 30 promote CH_4 production. Further analyses are currently undergoing to determine whether presence or absence of BSFL have modified the physico-chemical nature of our horse feces and consequently the quantity of CH_4 emissions.

Effects of black soldier fly (*Hermetia illucens*) by-product application on the whole-cycle performance of fungus gnats (Diptera: Sciaridae)

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As production of fishmeal, being a feed source for the growing aquaculture sector, is declining due to the consequences of overfishing, rearing of the black soldier fly (BSF; *Hermetia illucens*) for producing substitutes is on the rise. Thus, by-products of BSF rearing (frass, pupal cases, imagines) will make up considerable amounts as the sector grows, and the use as organic fertilizer is a promising option for the valuation of these side streams. Chitin is an essential component of fungi and insects and is contained in the mentioned BSF by-products as well. Soil amendment with chitin has been observed to increase chitinolytic activity of the soil microbial community, which might affect soil-living stages of agricultural pests.

In this study, fungus gnats, a horticultural pest feeding on soil-living fungi and plant roots, were exposed to potting soil amended with different BSF by-products (native/autoclaved frass; frass mixed with pupal cases and imagines), and their performance (substrate choice, duration of development, and mortality) was assessed in a series of experiments. To clarify the observed effects, the substrate mixtures were incubated to monitor the time course of chitin content and chitinase activity.

Soil amendment with BSF by-products reduced the generation time of fungus gnats, which might be a consequence of increased growth of fungi, being their main feed source. Despite their shorter generation time, population size was greatly reduced compared to pure potting soil. As the autoclaved treatment performed worse, effects may not only be directly related to chitin but may also depend on microbial activity.

With BSF by-products being effective against fungus gnats, they could be included into horticultural potting substrates. Effects on other soil-living organisms and its further potential in integrated pest management might need to be assessed as well.

Biovalorization of digested municipal sewage sludge: *Hermetia illucens* vs *Tenebrio molitor*

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Digested municipal sewage sludge is a waste that is created after methane fermentation of excessive active sludge in the wastewater treatment plan. It can be used as fertilizer on arable soils but it must meet environmental requirements as to the content of heavy metals. Therefore, its use is not easy. We used digested municipal sewage sludge as feed for *Hermetia illucens* and *Tenebrio molitor* larvae. The hypothesis assumed that the larvae of these insects would be able to biovalorise it and utilize the waste as a whole, which is important in the entomoremediation context. Experimental variants were 6 doses of the sludge: 25, 50, 75, 100, 500 and 1000 mg dry weight (DW) of the sludge on each larvae. The used sludge was wet and had ca. 20% of DW, thus its wet amount had been calculated to obtain appropriate content of dry weight in each experimental variants. The utilization of the DW of the sludge by *H. illucens* larvae was in the range of 14.6 ± 0.5% in 500 mg variant to 21.1 ± 5.4% in 1000 mg variant. Contrary to *H. illucens*, *Tenebrio molitor* larvae utilized the sludge the best in the lowest dose variant: the percent of utilization was the highest in 25 mg and was 82.1 ± 1.9% DW. Only 7.8 ± 0.8% DW utilization was recorded in 1000 mg. The research presented in the paper was partially financed by the Poland National Science Centre, as a result of the project no. 2019/35/D/NZ9/01835.

Frass to gas: industrial insect rearing residues as co-substrates for anaerobic digestion

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Large-scale insect farming has experienced noticeable growth over the last years, with the recent endorsements by the EU's legislative framework further leveraging the industry's development. Among the few species authorized for their use in food or feed, the black soldier fly (BSF) may be considered the most versatile. An estimated annual growth of 35% predicts a market volume of more than $ 3 billion by 2030 for this species. The required upscaling of production following the enactment of appropriate legal guidelines could thus be able to produce more than 5 million tons of insect protein per year by 2030.

Consequently, large amounts of insect rearing residues will inevitably be generated. This mixture of undigested substrate, shed cuticles, and insect excrements is currently promoted as effective organic fertilizer suitable to complement or even fully replace mineral fertilizers. However, in cases where this frass is unfit for soil application or the marketing as fertilizer is unviable, sustainable treatment solutions are needed.

Here, we present insights into the applicability of BSF frass as co-substrate for the anaerobic digestion of cattle slurry. The frass was generated by the conversion of agro-industrial by-products in large-scale feeding trials. Within the period of two hydraulic retention times, we successively applied increasing concentrations (2, 4, and 6%) of frass and monitored daily biogas yields and quality. Two control groups running on either pure slurry or on a co-substrate consisting of the frass' original substrate prior to larval conversion served as points of reference.

With average concentrations of 51 ± 2% and 41 ± 2% for CH_4 and CO_2, respectively, gas quality was comparable across treatments and throughout the experiment. Frass addition, however, induced an increase of biogas yields of up to 70% at the highest co-substrate concentration. Comparing these yields with the biomethanisation of the unprocessed larval substrate indicated that, even after larval bioconversion, these residues still constitute a suitable resource to enhance biogas production from agricultural wastes. Therefore, frass may not only serve as valuable fertilizer, but could also contribute to cover the energy demands by industrial insect farming.

Frass from black soldier fly larvae as a valuable fertilizer and biopesticide for crops

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Insect farming has gained recognition globally and has a number of advantages when implemented at the small and large scale. Recent studies, especially on the black soldier fly, *Hermetia illucens*, have shown that insects can be farmed and utilized as human food and animal feed. However, the byproduct of insect feeding, a combination of food residue and frass left by the insects after harvesting, has not been given much attention and only a few studies have managed to conduct research in using the byproduct as a bio-fertilizer. In sub-Saharan Africa, human population is expected to double, and most people depend on farming for survival; however, food prices are increasing, and fertilizers are expensive for local farmers. Using frass produced by insect farming follows closed loop economic principles that will reduce waste, recover nutrients and improve livelihoods. The main objectives of this project are (a) to evaluate the quality of frass as an organic fertilizer and determine whether the frass allow pest colonization in the *Amaranthus hybridus* (Amaranthus plants). Preliminary results indicate that commercial fertilizers remain superior in terms of plant growth. However, frass treatment level of 20 g in a 5 kg pot of soil had good plant growth after commercial fertilizers, while the chlorophyll content was higher for plants grown in the 20 g treatment. Therefore, frass can be a decent substitute for agricultural purposes Conclusions can be made on frass quality but more studies are required to determine the quality of frass depending on the waste used to feed the larvae.

An economic purification platform for insect-derived antimicrobial peptides

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The overuse and inappropriate application of antibiotics has led to the advent of multi-drug-resistant pathogens. An effective treatment of infections caused by these pathogens is becoming increasingly difficult, particularly as the development of new antibiotics has slowed down since the late 1990s. An alternative therapeutic approach, suitable to overcome the resistances to common antibiotics, is seen in antimicrobial peptides (AMPs). As a part of the innate immune system of many organisms, AMPs possess a broad natural activity spectrum against viruses, bacteria and fungi. Due to the large biodiversity of insects, they are very well suited for screenings for potential AMP candidates. Furthermore, their field of application is not limited to therapeutic applications. AMPs also play an essential role as preservatives in the food, feed and cosmetic industry.

For their industrial application, large quantities of AMPs need to be produced either chemically, or in the case of larger and more complex AMPs, biotechnologically. Therefore, an economic and efficient production process must be developed, which is also addressed in our presented work. We focus on a downstream platform technology, based on small-molecule fusion tags, which facilitates the purification of AMPs, while potentially enhancing their upstream expression levels.

We demonstrate the successful use of the commercial C-tag (EPEA) for the production and purification of a model AMP: Insect metalloproteinase inhibitor (IMPI). The C-tag, does not interfere with the AMPs' activity, thus, it is not necessary to remove the tag from the AMP, which improves the overall process economics.

Termites farming: a new horizon to bioconvert lignin-rich residues into high-value products

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Termites have a tremendous ability to digest lignocellulosic materials and their recalcitrant constituents, which is unequalled into the entire animal kingdom. The present work, belonging to the European BBI-JU project ZELCOR, proposes to exploit this termites' conversion potential as a promising approach to produce chitin and its derivative chitosan. While termites are traditionally harvested from the wild for food or traditional medicine in many parts of the world, one of the most important aims of this projet is for the first time to rear termites at an industrial scale. Farming termites is extremely challenging especially because these insects are organized into complex societies, socially structured, capable of construction and therefore hardly processable. While addressing this complexity, Ÿnsect and UPEC have developed disruptive solutions to fix the main bottlenecks at stake. Firstly, the ability of termites to be fed on technical lignin substrate from biorefineries was established. The changes in the ratio of syringyl and guaiacyl units in lignin and the structure of bacterial gut microbiota were assessed by thioacidolysis analysis, and 16S rRNA amplicon sequencing on the MiSeq platform from Illumina, respectively. Secondly, methods for termites withdrawal and processing were developed. A new chitin extraction process was set up to obtain high-quality products comparable to mealworms based on molecular and structural similarity. Thirdly, by considering the termite colony as a superorganism, a mathematical prediction model was developed based on matrix calculation to determine the production and reproduction parameters of a termite colony that would allow viable industrial breeding.

This significant progress shed light on the feasibility of controlled farming of termites, often considered as impossible, opening new territories to the insect industry towards the high valorization of lignin-rich side streams.

Competitive insect products Feedstock suitability assessment for *Hermetia* rearing and waste treatment of insect farming residues by anaerobic digestion

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In the research project “Competitive Insect Products (CIP)”, the suitability of various organic feed materials for larval rearing of the black soldier fly has been tested in laboratory- and pilot-scale feed tests. Larval growth and weight gain were investigated under controlled conditions over a period of 2–3 weeks in batch operation. In addition to numerous raw materials of feed quality such as brewer's grains, residues from bioethanol production, maize silage, agricultural residues such as fermentation residues from agricultural biogas plants, dry chicken manure as well as residues from watercourse management were investigated. Based on the results of the feeding trials, an initial estimate of the required feed input quantities and the substrate-side production costs has been made. Depending on the feed material used and the feeding rate selected, a conversion of the organic dry matter (oTS) contained in the feed material into insect biomass (oTS) of between 4...29% could be achieved. Depending on the process control, the residues of larvae fattening are comparatively dry (70...85% dry matter).

A further aim of the CIP project was to determine the biogas potential and the fermentation properties of residues from insect farming (insect frass). For this purpose, discontinuous fermentation tests and quasi-continuous fermentation tests were carried out in lab scale. The specific gas potential of the insect frass samples from lab feeding trials was in the range of 168...288 mL CH_4 / g organic total solids (oTS) and was thus comparable to other animal residues such as cattle, pig and chicken manure with 210, 250 and 280 mL/g oTS, respectively. Insect frass from *Hermetia* rearing in pilot scale achieved methane potentials of approx. 168 mL CH_4 / goTS or 118 m^3 CH_4 / t residue in long-term fermentation tests on a laboratory scale, which corresponds to the typical methane potential of maize silage with approx. 110 m^3 / t fresh mass. Accordingly, one tonne of maize silage could be replaced by the use of one tonne of insect frass from insect farming.

The poster or presentation will summarize the main project results and will give an outlook towards the current research work on insect farming.

The impact of genotype-environment interactions on the microbiota in the larvae of the black soldier fly (*Hermetia illucens*)

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The black soldier fly (*Hermetia illucens*) has taken the centre stage in the industrial insect rearing sector, in part due to the ability of its larvae to convert a broad range of substrates into protein-rich biomass. With the industrial importance of this insect on the rise, fundamental research questions are being explored as well to standardize rearing methods, improve waste degradation and maximize biomass output. One example is the exploration of which factors determine the substrate-dependent efficiency of larval growth. One factor that is known to interact with the substrate is the microbiome in the larval gastrointestinal tract (GIT). It is still under debate whether this interaction then drives the observed variation in growth efficiency. To solve this question a fundamental understanding is needed of how the microbiome is shaped in the GIT. Here, we explore the interaction between the genotype, the substrate and the GIT microbiome composition. Four different genotypes, from the collection recently described by Kaya et al. (2021), were reared on three distinct substrates (chicken feed, preconsumer food waste and chicken manure). At the end of the rearing cycle, the microbial composition was determined for both larvae and residue using 16S rRNA gene sequencing. Additionally, quantitative real-time PCR (qPCR) has been performed as well to transform relative into absolute abundances. Briefly, our results reveal that in general the same zOTUs are identified for all the larvae of the different genotypic lines reared on the same substrate, yet the abundance of specific zOTUs varied between genotypes. For the residue, a clear correlation between the microbiome composition and the substrate used is observed, yet at the same time the occurrence of specific zOTUs can be correlated to specific genotypes. Future research should explore by which mechanisms the genotype affects the microbiome, e.g. by differential expression of certain antimicrobial peptides.

Experimental Investigations about the dietary protein requirement of the yellow mealworm (*Tenebrio molitor*)

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Intensive research about insects for food and feed has been done in recent years, but nutrient requirements of insects remained unnoticed. So, the aim of the present study was to determine the crude protein (CP) requirement of *Tenebrio molitor*.

50,000 growing mealworms (av. bodyweight: 40 mg) were allocated to 50 groups with about 1000 animals per group. They were kept in plastic bowls at 28 °C and 50% relative humidity. 9 isoenergetic diets with CP contents of 9.0, 11.5, 13.9, 16.3, 18.6, 20.6, 23.0, 25.7, or 28.0 g per 100 g dry matter (DM) were formulated using wheat flour, starch, gluten, oil, and a vitamin-mineral mix. A commercial layer feed (22.7 g CP per 100 g DM) was the positive control diet. Each of the 10 diets was given to 5 mealworm groups. Feed supply (FS) to each group was adjusted daily according to visual judgement of feed consumption. Biomass (BM) of mealworms and feed supply (FS) were recorded per bowl every 5 days starting with experimental day 10. On day 31, mealworms were euthanized by freezing. Weight gain (WG), final BM, and total FS were statistically analysed using ANOVA (all 10 diets) as well as regression analysis (9 experimental diets) using dietary CP concentration as regression determinant.

Total FS, final BM, and WG responded to rising dietary CP concentrations according to a cubic function (R^2=0.99) with increasing values at very low dietary CP and maxima at around 12.0 g CP per 100 g DM, followed by a decrease at further rising CP concentrations ($p<0.01$). Growth performance with control (layer) diet containing 22.7 g CP per 100 g DM and supplemented essential amino acids was equal to the diet containing per 100 g DM only 11.5 g CP of putatively low quality (predominantly gluten).

This gives rise to the hypothesis that CP requirements of mealworms are lower than currently assumed. The depressive effects of the high dietary CP contents probably was due to physiological reasons (e.g. amino acid imbalances) or/and physical problems (e.g. sticky consistency of feeds). In total, protein requirements of insects in terms of dietary concentrations as well as amino acid composition need to be investigated more in detail as the experimental results derived from common livestock species might not be appropriate for insects.

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Heft 69	Image Analysis for Agricultural Products and Processes 27 to 28. Aug. 2009 Potsdam	2009
Heft 70	5th International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management 31. Aug. to 02. Sept. 2009 Potsdam	2009
Heft 71	Einsatz von Biogas in PEM-Brennstoffzellen	2009
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Heft 73	16. Workshop Computer-Bildanalyse in der Landwirtschaft 04. Mai 2010 Braunschweig	2010
Heft 74	Erschließung von Nachhaltigkeitspotenzialen durch Nutzung innovativer Sensortechnologien – *Prozesskette Getreide* –	2010
Heft 75	Erschließung von Nachhaltigkeitspotenzialen durch Nutzung innovativer Sensortechnologien – *Prozesskette pflanzliche Frischeprodukte* –	2010
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Heft 77	A flow cytometric approach to monitor the effects of gentle preservation techniques in the postharvest chain	2011
Heft 78	17. und 18. Workshop Computer-Bildanalyse in der Landwirtschaft 05. Mai 2011 Stuttgart und 09. Mai 2012 Osnabrück	2012
Heft 79	2. Öffentliches Symposium des „BCN“ BiogasPOTENZIALE Erkennen, Erforschen, Erwirtschaften	2012
Heft 80	Mechanisms of Bacillus spore germination and inactivation during high pressure processing	2013
Heft 81	19. Workshop Computer-Bildanalyse in der Landwirtschaft 2. Workshop Unbemannte autonom fliegende Systeme in der Landwirtschaft 06. – 07. Mai 2013 Berlin	2013

Heft 82	3rd Global Workshop on Proximal Soil Sensing	2013
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Heft 84	Prozessmikrobiologie in landwirtschaftlichen Biogasanlagen Schlussbericht zum Forschungsverbund BIOGAS-BIOCOENOSIS	2014
Heft 85	Sensoren.Modelle.Erntetechnik Kolloquium zur Verabschiedung von Dr. Ehlert 27. Mai 2014, Potsdam-Bornim	2014
Heft 86	Phosphor für die Landwirtschaft – Strategien für eine endliche Ressource 11. Juni 2014, Potsdam-Bornim	2014
Heft 87	Biofilme in Biogasanlagen – Struktur, Einfluss auf die Biogasausbeute und Optimierung technischer Systeme zur Rückhatung der mikrobiellen Biomasse BIOGAS-BIOFILM	2015
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Heft 90	ISHS Symposium 2016 “Sensing Plant Water Status” Methods and Applications in Horticultural Science 05th – 07th October 2016 Potsdam	2016
Heft 91	10Th International FRUTIC Symposium Quality and Safety of Fresh Horticultural Commodities February 07, 2017	2017
Heft 92	Etablierung eines *core*-Mikrobioms für Biogasanlagen Genom-Sequenzierung von Isolaten aus Biogasanlagen und Mapping von Metagenom-Datensätzen BIOGAS-CORE	2017
Heft 93	22. Workshop Computer-Bildanalyse und Unbemannte autonom fliegende Systeme in der Landwirtschaft 23. Workshop Computer-Bildanalyse in der Landwirtschaft 21. April 2016, Wernigerode und 27. April 2017, Potsdam-Marquardt	2017
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Heft 97	INSECTA Conference 2017 07th – 08th September 2017, Berlin, Germany	2017
Heft 98	Storability of broccoli – investigations of optical monitoring, chlorophyll degradation and predetermination in the field	2018
Heft 99	24. Workshop Computerbildanalyse in der Landwirtschaft 25. April 2018, Zürich	2018
Heft 100	INSECTA 2018 05th – 07th September 2018, Giessen, Germany	2018
Heft 101	6th International Conference on Machine Control and Guidance 1st – 2nd October 2018, Berlin, Germany	2018
Heft 102	25. Workshop Computerbildanalyse in der Landwirtschaft 17th 2019, Bonn, Germany	2019
Heft 103	INSECTA 2019. International Conference 05th – 06th September 2019, Potsdam, Germany	2019
Heft 104	Transformation Strategies in Agriculture	2021
Heft 105	INSECTA 2021. International Conference 08th – 09th September 2021, Magdeburg, Germany	2021

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Paper edition: 20,00 Euro

Internet (open access): http://www.atb-potsdam.de/bab