Upcycling of mycotoxin-contaminated grains to food:
The Yellow Mealworm (*Tenebrio molitor*), a safe utilizer of trichothecene-contaminated oats?

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Meinen Eltern

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List of abbreviations

A(C)	Artificial Control
ACN	Acetonitrile
A(LD)	Artificial Low Dose
A(HD)	Artificial High Dose
EC	European Commission
EU	European Union
DON	Deoxynivalenol
HT-2	HT-2 toxin
HPLC	High Performance Liquid Chromatography
IC_{50}	Half maximal inhibitory concentration
IPIFF	International Platform of Insects for Food and Feed
LOD	Limit of Detection
LOQ	Limit of Quantification
MS	Mass Spectrometer
MTT	Methylthiazoltetrazolium
N(C)	Natural Control
NIV	Nivalenol
N(LD)	Natural Low Dose
N(HD)	Natural High Dose
Novel Food Reg.	Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods
Reg.	Regulation
SCF	*Scientific Committee on Food*
SCOOP	Scientific Co-operation on Questions relating Food
T-2	T-2 toxin
Triol	T-2 triol
t-TDIs	Temporary-Tolerable Daily Intakes
Tetraol	T-2 tetraol

1 Introduction

With over one million described and up to 2.5 million estimated species (Stork, 2018; Zhang, 2011), insects are the most diverse animal group on earth. They are abundant on all continents and oceans of the world and appear in most diverse habitats, such as the glaciers streams of the Alps (Fuereder et al., 2001), the canopy trees of New Guinea (Novetny et al., 2002) up to the shores of Japan (Ikawa et al., 2004). But it is not only their taxonomy and behaviour that have been research topics for decades, also the interest in their economic importance has strongly increased and spread throughout various topics.

The fact that edible insects have been stated to be a potential source to reach the first three United Nations Sustainable Development Goals (no poverty, zero hunger, good health and well-being) (FAO, 2009) has boosted scientific interests regarding not only farming and processing, but also consumer acceptance, nutritional value and safety aspects (Imathiu, 2019; Montowska et al., 2019). However, whereas insects as food are already well known and part of the local diet in regions of Africa, South America and Asia, entomophagy is quite unfamiliar in Europe and North America and can be met with refusal (Elhassan et al., 2019; Raheem et al., 2019; Tan et al., 2015). Especially in Europe, concerns about appearance and safety of insect products lead to refusal or even disgust (Bednářová et al., 2013; Hartmann et al., 2015; Yen, 2009).

The economic value of insects has highly increased, particularly in Europe. As a result, some species such as the Black Soldier Fly (*Hermetia illucens*) and the Yellow Mealworm (*Tenebrio molitor*), formerly seen as pests, are now recognised as products of high value for food and feed (Tomberlin and van Huis, 2020; Tomberlin et al., 2015) and their economic and ecological value is part of ongoing research (Kierończyk et al., 2019; Manzano-Agugliaro et al., 2012; Yen, 2015).

In order to dissipate these existing concerns various food safety aspects, such as microbiological hazards (Klunder et al., 2012) or unwanted residues, for instance pesticides or heavy metals (Poma et al., 2017) have been intensively investigated within the last decade. As a result, safety schemes for farming and processing were derived and implemented into European guidelines (IPIFF, 2019).

Recent studies indicated that insects – if kept on rotten and/ or mouldy substrates – may render hazardous metabolites harmless via their own metabolism or largely excrete them, especially mycotoxins, such as zearalenone or type B trichothecenes (Niermans et al., 2019; Ochoa Sanabria et al., 2019; Van Broekhoven et al., 2017). Based on these studies, there could be the possibility to obtain a safe product for further use for food or feed through insects reared on material no longer suitable for animal or human consumption. However, as shown for zearalenone, metabolism in *T. molitor* may also result in formation of compounds of higher toxicity, such as α-zearalenol (Niermans et al., 2019).

Various insects species appear to have different metabolisation and excretion pathways for mycotoxins, as described for aflatoxin B1 (Bosch et al., 2017) or type B trichothecenes and ochratoxins (Camenzuli et al., 2018). However, comprehensive investigations on the metabolism and fate of trichothecenes in *T. molitor*, especially type A trichothecenes, are not available so far. In general, type A trichothecenes show several adverse effects, e.g. cytotoxicity and leukopenia in mammals (Bauer et al., 1989; Li et al., 2011), or apoptosis and disturbed protein biosynthesis (Shifrin, 1999; Thompson and Wannemacher Jr, 1990). Furthermore, the abundance of type A trichothecenes in various cereals for food and feed have been investigated and several detection methods have been established (JECFA, 2001).

Therefore, edible insects could be a high-value and safe food or feed alternative, if not accumulating trichothecenes. Especially as utilization of grains, containing high mycotoxin amounts of natural origin affected the growth rate of *T. molitor* positively (Niermans et al., 2019).

The aim of present study was to examine the ability of *T. molitor* larvae as safe utilizers of oats contaminated with T-2 and HT-2 toxins. In order to investigate possible effects of mycotoxigenic fungi and type A trichothecenes on the growth and health of *T. molitor*, larvae were kept on naturally moulded and artificially contaminated oats. Additionally, the occurrence of T-2, HT-2, T-2 triol and T-2 tetraol in the larvae and their residues was determined via LC-MS/MS analysis.

2 Literature

2.1 Edible Insects as Food

2.1.1 Entomophagy and nutritional values of insects

Insects are the most diverse animal groups on earth, with over one million described and up to 2.5 million estimated species (Stork, 2018; Zhang, 2011). According to Jongema (2017) entomophagy, the use of insects as food, is practised worldwide (see. Fig. 1) and for about 2 billion people insects are part of their diets (Van Huis et al., 2013). In many countries, such as Zimbabwe, Mexico and Thailand, edible insects are well known, whether as basic food or as delicacy (Raheem et al., 2019).

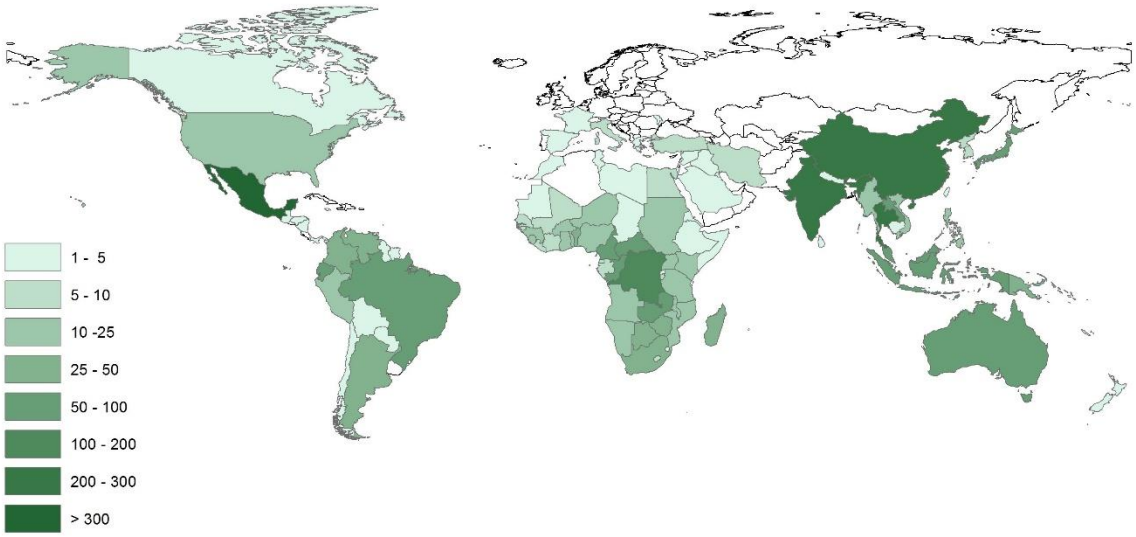

Figure 1 Recorded edible insects species, by country. Grenisch levels indicate number of edible insects in respective country. (Jongema, 2017)

However, whereas edible insects are part of the local diet in regions of Africa, South America and Asia, entomophagy is quite unfamiliar in Europe and North America and can be met with refusal (Elhassan et al., 2019; Raheem et al., 2019; Tan et al., 2015).

Also in countries like Australia, where entomophagy is known through native culture, concerns about appearance and safety of insect products are leading to refusal or even disgust in parts of the society (Yen, 2009). Especially in Europe, edible insects are generally not considered as appropriate food and so food neophobia and disgust can lead to an indefinite refusal of novel foods (Hamerman, 2016; Tan et al., 2016). However, as stated by Bednářová et al. (2013) and Hartmann et al. (2015) the European consumer acceptance can be increased by the usage of

known edible insects species, like *T. molitor* or *Locusta migratoria* and if insects are incorporated into familiar food items, such as burgers or noodles. Therefore, it could be helpful to adapt insect-based food to European preferences concerning flavour, texture, and appearance.

Nowadays more than 2100 species are referred as edible (Jongema, 2017) and nearly 1700 insect species are documented as being used as human food (Ramos-Elorduy, 2006). The most commonly eaten are insects from the orders Coleoptera (31%), Lepidoptera (17%), Hymenoptera (15%), Orthoptera (13%) or Hemiptera (11%) (see. Fig. 2).

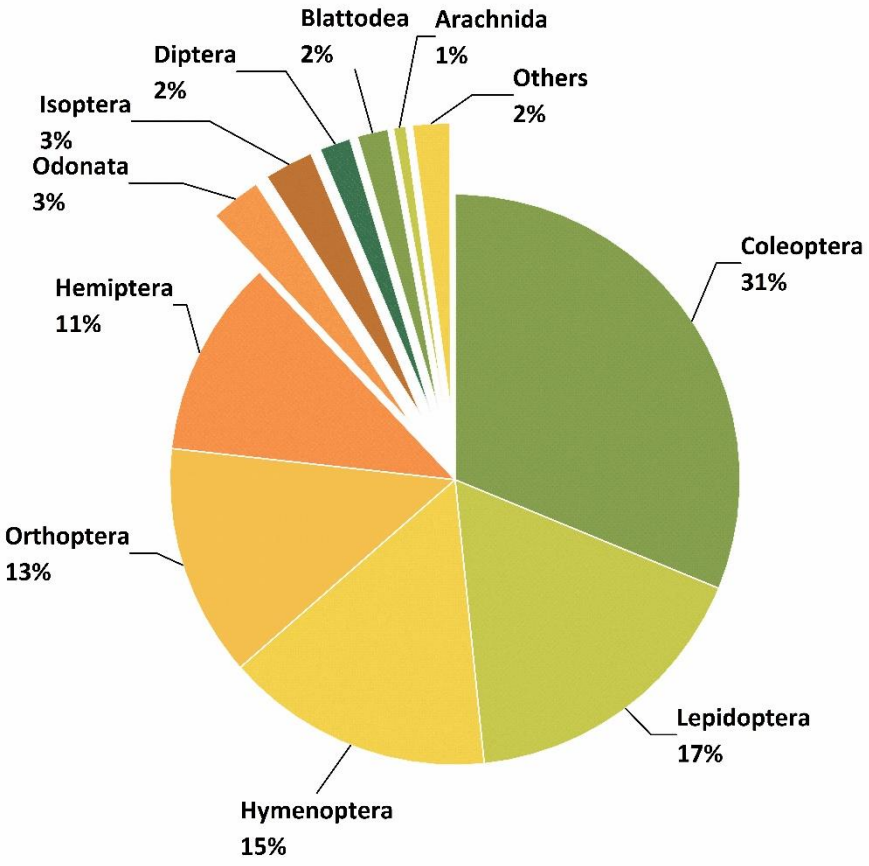

Figure 2 Worldwide percentage of edible insects and spiders (Adopted to: Jongema, 2017)

The Food and Agriculture Organization of the United Nations (FAO) stated edible insects to be a potential product or diet to reach the first three United Nations Sustainable Development Goals (no poverty, zero hunger, good health and well-being) (FAO, 2009).

Therefore, several insect species have been closely investigated and the results indicate that they meet the requirements for an adequate vertebrate diet, either to be used in human food or animal feed (e.g. for fish or chicken). For instance, crickets (*Acheta domesticus, Gryllodes sigillatus*), mealworm larvae (*T. molitor*) and adult locust (*Schistocerca gregaria*)

were demonstrated to be rich in energy, fat and protein as well as high levels of minerals such as calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) (see. Table 1) (Montowska et al., 2019; Zielińska et al., 2015).

Also Nowak et al. (2016) stated that the nutrient composition of *T. molitor* is sufficient for human consumption and could possibly reduce undernourishment. An important fact, as undernourishment still affects more than 800 million people worldwide according to FAO et al. (2019). Moreover, also the usage of insects as an alternative protein source is conceivable in animal feed, e.g. in fish aquaculture (Barroso et al., 2014) or for insectivores (Finke, 2002).

However, it remains crucial to take the specific requirements of the various vertebrate species into account. Additionally, the evaluation of the protein content of edible insects, usable as food and feed, is discussed controversially. Whereas the protein content of several insects ranges from 52 % to 76 % according to Zielińska et al. (2015), Jonas-Levi and Martinez (2017) state that these amounts could be overestimated due to the lack of appropriate measurement methods.

Table 1 Nutritional composition and energy content[a] of four edible insect species reared in the EU (compiled from Montowska et al. (2019); Zielińska et al. (2015))

Analytical Parameters	Species			
	Gryllodes sigillatus	*Acheta domesticus*	*Tenebrio molitor*	*Schistocerca gregaria*
Protein [%]	70 ± 1.7	44.2 ± 0.5	52.3 ± 1.1	76.0 ± 0.9
Fat [%]	18.2 ± 0.7	25.5 ± 0.5	24.7 ± 1.5	13.0 ± 0.7
Fiber [%]	3.7 ± 0.5	5.9 ± 0.3	2.0 ± 0.3	2.5 ± 0.3
Ash [%]	4.7 ± 0.4	4.0 ± 0.3	3.6 ± 0.6	3.3 ± 0.5
Carbohydrates [%]	0.1 ± 0.0	20.5 ± 0.7	2.2 ± 0.3	1.7 ± 0.2
Energy [kJ/100g]	1896 ± 12.5	2090 ± 89.1	1857 ± 15.2	1821 ± 11.2

[a] Based on dry matter; *Ash* corresponds to the mineral content of the sample

2.1.2 European Regulations and Guidelines

The Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods (Novel Food Reg.) entered into force in January 2018. This so-called “Novel Food Regulation” acting in accordance with the existing Regulation (EU) No 1169/2011, (European Union 2015). Article 3 paragraph 2 point a defines Novel Food, as any food that was not used for human consumption to a significant degree within the Union before 15 May 1997.

According to recital No. 8 of the Novel Food Reg. it is appropriate to review, clarify and update the categories of food, which constitute novel foods. In accordance to this stipulation the category "food from animals or their parts" (article 3 paragraph 2 point a (v)) is now considered to cover whole insects and their parts. Therefore, the Novel Food Reg. provides the legal basis for the placing of edible insects as food on the market within the European Union (EU). Insects currently reared within the EU should not have any adverse effects on plant, animal, or human health; they should not be pathogenic vectors for animals or plants and should not be under protection status or be defined as invasive alien species.

The following insect species are currently reared in the Union and comply with the above-mentioned safety conditions for insect production for feed use: Black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*T. molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*). Furthermore, according to Annex X of Regulation (EG) No. 142/2011 (European Union, 2011) as amended by Regulation (EU) 2017/893 (European Union, 2017) they have been authorised for the use as raw materials for feed for farmed animals.

Additionally, Annex XIV of Regulation (EG) No. 142/2011 (European Union 2011) states the conditions under which insect derived proteins may be imported into the EU, e.g. the substrates allowed for the feeding of the insects. These substrates may only contain products of non-animal material or category 3 material of animal origin, e.g. fishmeal, blood products from non-ruminants, eggs and egg products, honey or rendered fat.

Any kind of manure, catering or other waste may not be content of the substrate. The use of faeces and intestinal tract content as feed is also prohibited according to Annex III Regulation (EC) 767/2009.

Although some edible insects are considered as Novel Food already since 2018, concrete regulations regarding food safety and process hygiene criteria of edible insects have not been established by the EU yet.

A guide on good hygiene practices has been published 2019 by the International Platform of Insects for Food and Feed (IPIFF), referring to existing European legislation (IPIFF, 2019), including food safety and process hygiene criteria as well as recommendations for HACCP implementation into insect production.

In Switzerland, guidelines have been published through an information letter in 2017 (Beer, 2017). In these guidelines food safety and process criteria have been implemented for the three insect species Mealworm larvae (*T. molitor*), adult house cricket (*A. domesticus*) and adult migratory locust (*Locusta migratoria*), which are in Switzerland permitted for human consumption as whole animals as well as crushed or grounded state.

Thus, to ensure food safety of edible insect products, according to Regulation (EC) No 2073/2005 (European Community, 2005) as well as Article 3 (3) of the swiss hygiene regulation (HyV) (EDI, 2016) 25 g sample material have to be free of *Salmonella* spp. and have to contain less than 100 colony forming units *Listeria monocytogenes* per gram.

In order to fulfil the process hygiene requirements for edible insect products, several bacterial criteria, such as total count of aerobic mesophilic organisms, Enterobacteriaceae, coagulase-positive staphylococci and *Bacillus cereus* have to be investigated. For all guideline values see Table 2.

According to Annex Part C Regulation (EU) 68/2013 amended by Regulation (EU) 2017/1017, insects are allowed to be used as feed material (animal fat (number 9.2.1) or processed animal protein (9.4.1)) if they are not pathogenic to humans and animals in all their life stages (European Union 2017b). Furthermore, insects can now be used as processed animal proteins for aquaculture animals since the relevant feed ban of Regulation (EC) 999/2001 has been partially uplifted by Regulation (EU) 2017/893.

Yet, the use of processed insect protein is still not allowed in the feed of pigs and poultry, in order to prevent and control the spread of spongiform encephalopathies (Regulation (EC) 999/2001, Art. 7.2 and Annex IV Chapter 1 b (i)). In contrast, this prohibition is neither applied for the feeding of cats and dogs nor for the production feed intended for cats and dogs.

Table 2 Food safety and process hygiene criteria for edible insects in the EU (IPIFF, 2019)

Hazards	Main origins	Severity level	Management	Target	Limit	Reference
Aerobic microbiota 30°C	Hygiene indicator Manipulations	Low	Good Hygiene Practices	10^4 cfu/g	5x 10^4 cfu/g	REG EU 2073/2005 Section dry fruits
E. coli	Hygiene indicator Manipulations	Low	Good Hygiene Practices	10^1 cfu/g	5x 10^2 cfu/g	REG EU 2073/2005 Section dry fruits
Staphylococcus coagulase + (*S. aureus*)	Hygiene indicator Manipulations (raw materials or processing operations	Medium	Good Hygiene Practices	10^1 cfu/g	10^2 cfu/g	REG EU 2073/2005 Section dry fruits and Minced meat
Listeria monocytogenes		High	Sourcing/ breeding management	Absence in 25 g	Absence in 25 g	REG EU 2073/2005 Section ready to eat foods
Salmonella spp.	Insects intestinal tractus	High		Absence in 25 g	Absence in 25 g	REG EU 2073/2005 Section shellfish
Cronobacter spp. (*Enterobacter sakazakii*)	Insects	Medium	Sourcing/ breeding management	Absence in 10 g	Absence in 10 g	REG EU 2073/2005 Section infant formula
Bacillus cereus	Feed	Medium	Feedstock management	10^1 cfu/g	10^2 cfu/g	
Campylobacter spp.	Insect guts	Medium	Sourcing/ breeding management	Absence in 25 g	Absence in 25 g	
Moulds and Yeast	Insect guts	Medium	Good Hygiene Practices	10^2 cfu/g	10^3 cfu/g	

cfu Colony Forming Units

2.1.3 Economical perspectives and production data

The global population is expected to reach 9 billion people by 2050 and the FAO estimates that the world's food production has to increase up to 70% until that time to feed the world (FAO, 2009). This will consequently lead to an increase of competitions for resources between animal feed, human food, and fuel production. Therefore, farming edible insects for food and feed implies a possible solution for these upcoming challenges. Besides the above-mentioned nutritional benefits of entomophagy, edible insects have several economic and ecological benefits.

The fact that many insects are currently seen as pests in modern agriculture, proves their worldwide occurrence and the possibility to be reared in a more intense scale (DeFoliart, 1995; Panagiotakopulu, 2001; Tomberlin and van Huis, 2020). Farming insects as "mini-livestock" can result in lower ammonia and greenhouse gas emission compared to conventional livestock and requires much less farmland for rearing (Oonincx et al., 2010).
Moreover, as slaughtering, transportation and storage of pork and chicken contributes 17-25% to their total greenhouse emissions, processing and storing mealworms appears to be less resource demanding and more environmentally compatible (Oonincx and de Boer, 2012). Less water consumption is another advantage of insect farming. The water footprint (calculated as L/ g Protein) of mealworms, for instance, is about 30% lower compared to chicken meat and 80% lower compared to beef (Miglietta et al., 2015).

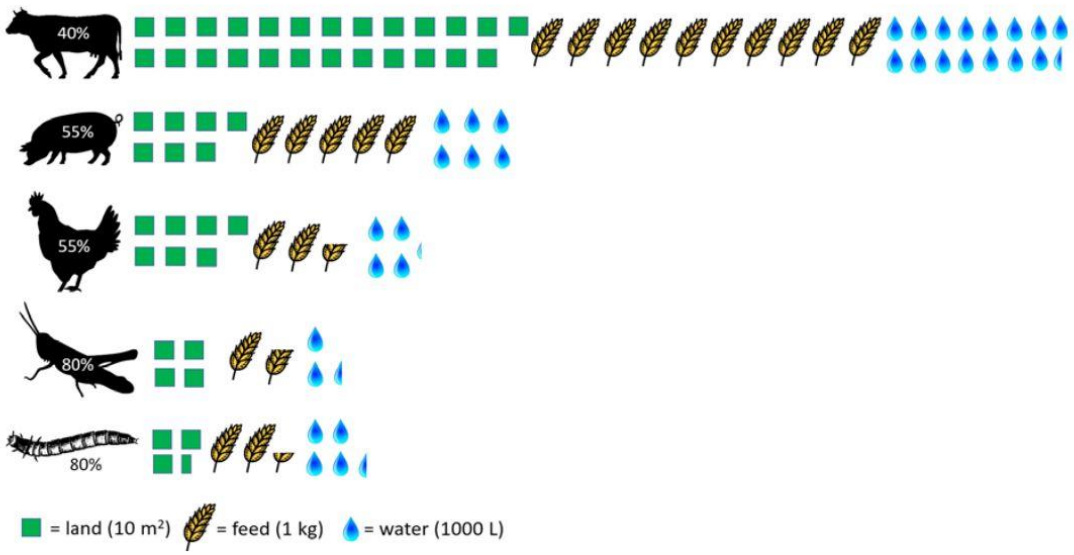

Figure 3 Amount of land, feed and water needed to produce 1 kg of live animal weight and percent of the animal which is edible (Dobermann et al, 2017).

Insects appear to have a very high bioconversion efficiency (i.e. the amount of feed needed to produce one kg of edible body weight) compared to over livestock animals (see Figure 3) (Dobermann et al., 2017; van Huis et al., 2015).
A possible explanation for this could be the poikilothermic physiology (internal temperature varies considerably, dependent on ambient environmental temperature) of insects (Van Huis et al., 2013). Insects do not need energy from feed to maintain their body temperature and can use it for building biomass instead. On the other hand, adequate environmental temperature thus is crucial for an overall-good development. In case of farming conditions, sufficient light supply has to be provided, in particular in most parts of Europe during winter season.

Not only the high bio conversion efficiency makes insects economical interesting, but also that up to 80 % of an insect is edible and digestible, compared to approximately 55 % for chicken or pigs and 40 % for cattle (Nakagaki and Defoliart, 1991)(see Fig.3).
As the industrial-scale insect production is relying on the same grain feed used for livestock, rearing of edible insects on low value organic by-products is discussed as another promising benefit. Lundy and Parrella (2015) investigated feeding of bio-waste as an environmentally viable way of insect production. In this study, crickets (*A. domesticus*) were fed with minimally processed food waste and diets composed largely of straw. Results showed a >99 % mortality of the crickets without reaching a harvestable size. Therefore, Lundy and Parrella (2015) recommend, instead of rearing insects on low organic by products, that the producers should attempt to use regionally available organic side-streams of relatively high-quality that are not currently being used for livestock production.

Although, all these production data appear to be very promising for economy and show that edible insects could be a sustainable food source for the fast-growing world population. On the other hand, most studies mentioned above have only been conducted on a small, extensive scale. However, extrapolation of the results to a large scale, intensive livestock farming of edible insects is limited. Therefore, more studies are needed, best in cooperation with countries experienced in insect farming, or huge insect food and feed producers in Europe.
A country with a large history of edible insect cultivation for the market or home consumption is Thailand, where 20,000 cricket farms produce an average of 7,500 metric tons of insects per year (Hanboonsong and Jamjanya, 2013).

In Europe, large-scale producers are sporadically located, like the Dutch company kreca®, which produces insects as food and feed for more than 40 years. However, concrete production data regarding edible insects in Europe are lacking.

Furthermore, insects cannot only be used to secure and complement food and feed chains, but also be part of developments in other promising business sectors. For example, the fatty acid methyl esters gained from *Chrysomya megacephala* (Fabricius) larvae fulfilled the requirements for raw material of biodiesel production (Li et al., 2012; Manzano-Agugliaro et al., 2012). Additionally, in times where plastic waste and micro plastic is challenging the worldwide waste systems and threatens the ecosystems, the search for insects as potential utilizers of plastic waste became part of current research. Therefore, mealworms (*T. molitor*) were demonstrated to possibly biodegrade and mineralize polystyrene (Yang et al., 2015a, b).

2.2 The Yellow Mealworm *(Tenebrio molitor)* as model organism

Yellow Mealworm is the name of the larval stage of the mealworm beetle (*Tenebrio molitor* (L)). *T. molitor* belongs to the family of the Tenebrionidae, which is one of the largest beetle families with more than 20,000 species worldwide and up to 1800 abundant species in Europe (Westheide and Rieger, 1996). The beetles of *T molitor* have lean bodies of brown or black colour, depending on their age, with a length of 12 - 18 mm (Westheide and Rieger, 1996). Female beetles are lacking an intersegmental membrane between 4^{th} and 5^{th} Abdominal sternite. The elytra are segmented through linear grooves and brown as well as the lower body, legs, and antenna. Their natural habitats are deserts, where they are able to gain water from the air through condensation on sand buildings or on their own. Nevertheless, like other Tenebrionidae beetles, they are a long known pest in agriculture (Panagiotakopulu, 2001), also occurring in storage units all over the world, especially in cereals.

Adult beetles use their environment for feeding, breeding and as cover during daytime. As the life stagesof *T. molitor* are temperature dependent, all further data are related to 25 °C. The life span of an adult female is about 90 days (Punzo, 1975). Initiate Oviposition takes place on the fourth day after imaginal moult, the eggs (about 500 per female) are scattered singularly or in groups, throughout foodstuff of high quality (Gerber, 1975; Gerber and Sabourin, 1984). The sticky, oval eggs have a diameter of 1.5 mm and are covered with dust and feed particles.

After 9 days, about 2 mm long larvae hatch of the egg. The white larvae live in the same environment as the adult beetles and are undergoing 12 - 14 moults in 119 ± 1 days (Ludwig and Fiore, 1960). The larvae feed themselves from vegetation (cereals), dead larvae, and moults between the larval stages and can reach a length of about 40 mm. After final moult they develop into pupae. The pupae are immobile and change their colour from white into brownish during the 9 days of holometabolic development. The total duration of *T. molitor* life cycle is 235 ± 2 days (Ludwig and Fiore, 1960).

The comparably low habitat and diet requirements of *T molitor* pledge these insects to be promising candidates for mini-livestock farming. The high amount of offspring and the short development cycles until harvest enable scientific investigations within an adequate time frame. The infrastructural requirements, such as available space, light, or temperature for farming, extensive as well as intensive, are affordable and in some regions of the world no artificial input (heating or light) is needed. These advantages of *T molitor* farming along with the above mentioned high nutritional values promise mealworm larvae to be a highly suitable model organism for scientific issues tackling food quality and/or security.

2.3 Trichothecenes

2.3.1 Classification and relevant moulds

Trichothecenes are a group of mycotoxins, containing over 200 metabolites and are produced by various genera of moulds such as *Fusarium*, *Myrothecium, Spicellum, Trichoderma, Cephalosporium and Stachybotrys* spp. Trichothecenes have a common tricyclic 12, 13-epoxtrichorhec-9-ene core structure (Cole et al., 2003; Ueno, 1984). They have been classified into four groups, type-A to type-D trichothecenes (see. Fig. 4).

Differentiation of the groups is based on the substitution at the C-8 position. Compounds of type A-trichothecenes have a hydroxyl group, an ester group (e.g., T-2) or no oxygen substitution at C-8. Type B-trichothecenes have a keto function at C-8, whereas type C-trichothecenes have a C-7/C-8 epoxide. Type D-trichothecenes are characterized through an additional ring linking the C-4 and C-15 position (see Fig. 4, (McCormick et al., 2011)).

Type- A and -B trichothecenes are the predominant natural contaminants in cereals and feed. T-2 toxin (T-2) - one of the most toxic trichothecenes- and its hydrolysed product HT-2 toxin

(HT-2) belong to type A trichothecene mycotoxins. A survey across Europe conducted by the Scientific Co-operation on Questions relating Food (SCOOP) revealed that 20 % of the analysed wheat samples were positive for T-2 toxin, whereas 14 % were contaminated with HT-2 (Schothorst and van Egmond, 2004). Gottschalk et al. collected between 2005 and 2006 a total of 289 samples of wheat products (n = 130), rye products (n = 61) and oat products (n = 98), of German origin. In total 85 % of all wheat product samples, 87 % of all rye product samples and 100 % of all oat product samples contained T-2. HT-2 was detected in 94% of all wheat product samples (Gottschalk et al., 2009).

T-2 and HT-2 are natural secondary metabolites formed by the worldwide-distributed moulds, especially of the genus *Fusarium.* Type A-trichothecenes are mainly produced by *F. langsethiae* and *F. sporotrichioides* in European cereals (Thrane et al., 2004; Torp and Nirenberg, 2004) growing on agricultural commodities on the field or during storage (Krska et al., 2001; Mirocha and Pathre, 1973).

Both human and animal livestock can be affected by *Fusarium* spp. by producing either mycotoxins or invasive diseases. A disease known as “alimentary toxic aleukia” with leukopenia, agranulocytosis, haemorrhagic diathesis, bone marrow aplasia and sepsis as symptoms, caused the dead of hundreds of thousands of people in the former Union of Soviet

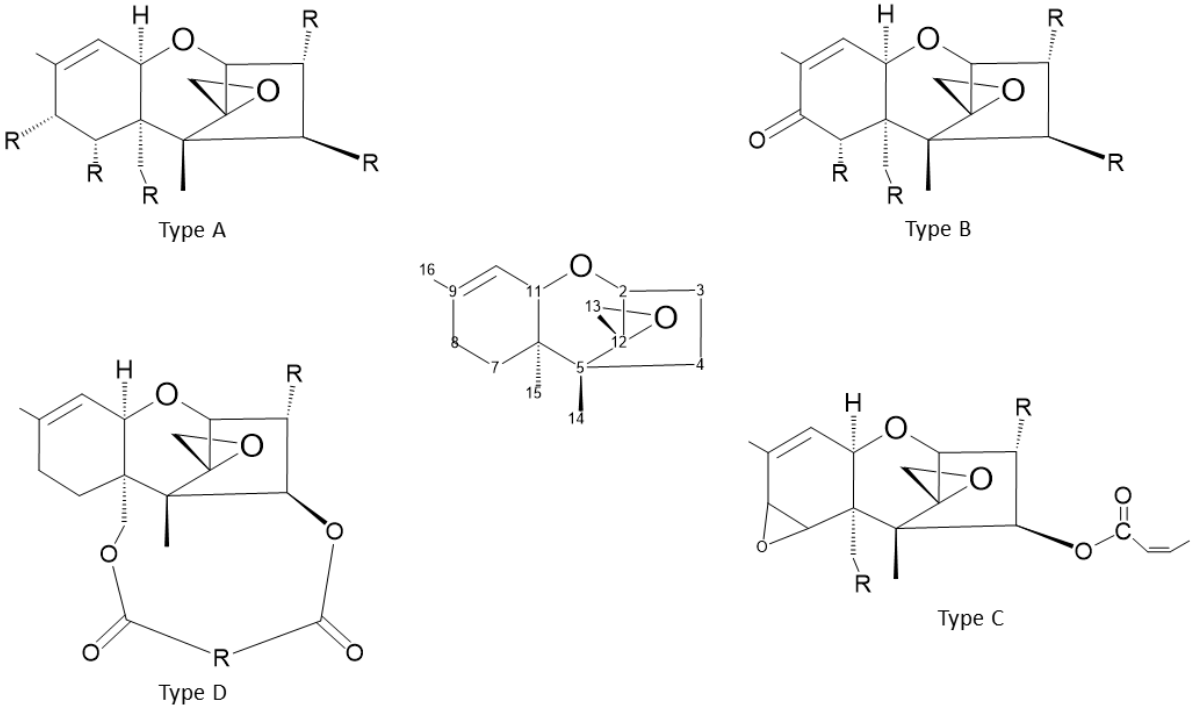

Figure 4 Trichothecene classification. R groups may be H, OH, OAcyl, or varations in the marcolid chain. (adopted to McCormick et al, 2011)

Socialist Republics during the second World War (Joffe, 1986). The occurrence of the disease was highly correlated to overwintered cereal grains colonized with *F. sporotrichioides* and *F. poae*, producing high amounts of T-2 (Mirocha and Pathre, 1973). Moreover, *Fusarium* species can cause rotting of roots, steams, ears as well as *Fusarium* head blight in wheat or other small-grain cereals all over Europe, resulting in severe reductions in crop yield (between 10% to 40%) (Bottalico and Perrone, 2002).

2.3.2 Metabolic pathway of T-2 toxin

After ingestion of contaminated food or feed T-2 toxin is metabolized extensively in the small intestine. As *in situ* experiments with jejunal loops of rats revealed, that only 2% of the injected toxin was recovered in plasma as unchanged T-2 after 50 min, while 25 % appeared as HT-2 toxin (see Fig. 5) in the effluent plasma. Furthermore, small amounts (<2% of the initial dose) of 3'-OH-T-2 toxin, T-2 tetraol and 4-deacetylneosolaniol were found (Conrady-Lorck et al., 1988).

Nearly the entire ingested amount of T-2 toxin is biotransformed in the organism (Cavret and Lecoeur, 2006). The mammal biotransformation of xenobiotics can be divided into two phases: Modification (Phase 1), in which reactive and polar groups are added to the substrate by diverse enzymes and Conjugation (Phase 2), hereby the now activated xenobiotics are conjugated with charged side groups in order to enable active transport and excretion (Jakoby and Ziegler, 1978). Hydrolysis, hydroxylation, and oxidation are the main reactions in trichothecene metabolism in animals. In the first phase, T-2 toxin is deacetylated to HT-2 toxin by the CYP450 esterases. These esterases show the highest activity in the microsomes of the liver, kidney and spleen (Ohta et al., 1977). For non- ruminants, the metabolic pathway of T-2 toxin in intestinal epithelium, kidney and liver involves two phases (Dohnal et al., 2008). Besides oxidation, the first phase also includes hydrolysis by non-specific esterases and amidases and reduction by epoxide-hydrolases (see Fig. 5). In the second phase, the metabolism is aiming for decreasing the toxicity of the substrates and increasing the water solubility of metabolites by conjugation reactions, mainly performed by glucuronosyltransferases (Dohnal et al., 2008). The conjugated metabolites are finally excreted, mainly by faeces (80%) and urine (20%) within 72h, as shown by Pfeiffer et al. (1988) with tritium-labelled T-2 toxin in rats.

Besides animals, plants and microbiota are also affected by trichothecenes and have evolved metabolic pathways. For instance, *Baccharis* spp. found in South America appear to be relatively tolerant to T-2 toxin. Additionally, macrocyclic trichothecenes, like roridins and verrucarins, are hydroxylated and transformed into other less toxic metabolites by these plant species (Dohnal et al., 2008).

Various species of yeasts, bacteria and filamentous fungi associated with the microbiome of cereal grains can extensively metabolise trichothecenes and have evolved several strategies to prevent the growth of *Fusarium* pathogens (Gdanetz and Trail, 2017; Pan et al., 2015; Wachowska and Głowacka, 2014). As described by Wachowska et al. (2017) these microbial strategies include modifications of trichothecenes, like acetylation, deacetylation, oxidation, de-epoxidation and epimerization and therefore lowers the pathogenic potential of these fungal secondary metabolites. As described by Yoshizawa et al. (1985) de-epoxidation of HT-2 and Tetraol by intestinal microbiota is part of type A trichothecene detoxification processes *in vivo*.

The metabolic pathway of trichothecenes in insects is not described, yet. As the class of insects, in particular the beetle family Tenebrionidae, have a different anatomy and physiology compared to mammals, it is to be assumed that the degradation pathway of trichothecenes is also different. Especially, since insects are able to detoxify - natural and synthetic - noxious agents in a way that is impossible for mammals (Bass et al. 2015; Ivie et al. 1983).

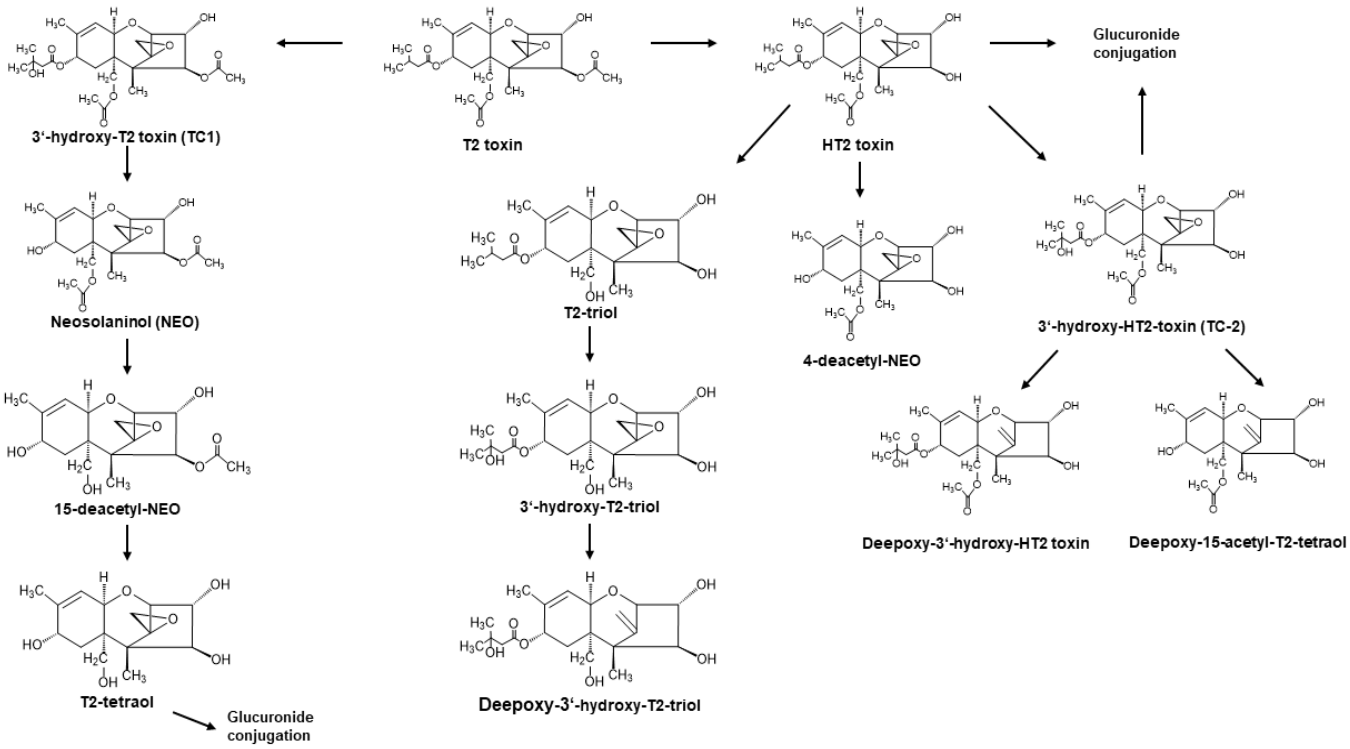

Figure 5 Metabolic pathways of T-2 toxin in mammals (adopted to Dohnal et al., 2008)

2.3.3 Toxicity of trichothecene similar substances on insects

As mentioned before, the metabolic pathway regarding xenobiotics is presumably different compared to mammals and most likely heterogeneous in the diverse insect families.

Bass et al. (2015) reviewed the biochemical and molecular mechanisms of insects involved in the resistance against neonicotinoid insecticides, one of the most important and worldwide used chemical classes of insecticides. Metabolic adaption appears to be the most common way of resistance. As shown for the silverleaf whitefly (*Bemisia tabaci* (L.)) neonicotinoid resistance is mainly conferred by enhanced detoxification by microsomal monooxygenases and overexpression of P450 cytochrome, CYP6CM1 (Karunker et al., 2008; Nauen et al., 2002; Rauch and Nauen, 2003). Regarding imidacloprid, one of the early neonicotinoid insecticides, there are 539 reported cases of resistance in 26 insect families according to the Arthropod Pesticide Resistance Database from 2000 until 2020 (APRD, 2020).

Insects have also evolved resistance mechanism to plant feeding deterrents. Various plant species produce organic chemical compounds, e.g. psoralens, in order to act as deterrents against insects or mammal herbivores. These psoralens (linear furocoumarins) are photoactive compounds that readily alkylate DNA when activated by longwave ultraviolet light and pose significant toxicological risks to man and other organisms. The insect family of the *Papilionidae* is able to feed on psoralen-rich plants due to their highly efficient capacity to detoxify these chemicals. Almost all detoxification processes occurring prior to absorption in the midgut tissue (Ivie et al., 1983).

Antibiotics are considered as xenobiotics in mammals as well as in insects. Therefore, antibiotic effects on insect physiology could be used for comparison of mammal and insect metabolic pathways. Li et al. (2020) showed that two commonly medical used antibiotics - chloramphenicol and vancomycin- exerted substantial effects on silkworms (*Bombyx mori* (L.)). As shown in this study the used antibiotics decreased the antioxidant enzyme activities and caused oxidative damage to silkworm intestine, effects also described in mammal tissue (Kohanski et al., 2017). Actinomycin D and mitomycin C, both cytostatic antibiotics, prevent imaginal differentiation in *T. molitor* pupae (Socha and Sehnal, 1972). Blockage of apolysis and retention of pupal features in emerging adults have been observed, clearly showing cytotoxic effects of actinomycin D and mitomycin C on insect morphogenesis. Both case studies showed comparable metabolic processes in mammals and insects regarding antibiotics, but detailed biotransformation pathways have not been described yet for insects.

2.3.4 Toxicity of type-A trichothecenes in humans and animals

Type-A trichothecenes can affect diverse parts of mammal physiology, e.g. nutrient uptake, DNA and protein biosynthesis, apoptosis, and alterations in the cellular membranes. Furthermore, they are seen as highly cytotoxic and immunomodulatory. Known effects of T-2, a major type A trichothecene, concerning selected physiological effects are listed in Table 3 below.

Table 3 Effects of T-2 toxin on mammal physiology (adopted to JECFA, 2001)

Location of effect	Effect	Species	Reference
Apoptosis	Apoptosis induction by ribotoxic stress	Human (Jurkat T Cells)	Shifrin (1999)
	Protein Kinase C induced apoptosis of hematopoietic and lymphoid tissue	Mice and Rat	Doi et al. (2006)
Cellular membrane	Increased lipid peroxidation	Duck, Chicken and Goose	Mezes et al. (1999)
DNA biosynthesis	Inhibition of thymidine incorporation	Human (*in vitro*)	Munsch and Müller (1980)
	Suppression of DNA synthesis	Rat	Thompson and Wannemacher Jr (1990)
Nutrition	Reduction of monosaccharide absorption	Rat	Kumagai and Shimizu (1988)
	Reduced vitamin E uptake	Chicken	Coffin and Combs Jr (1981)
Protein biosynthesis	Inhibition and Malalignment of proteins	Rat	Thompson and Wannemacher Jr (1990)
	Ribosomal peptidlytransferase inhibition	Human (Jurkat T Cells)	Shifrin (1999)

The biological activity of the various trichothecenes is based on their different functional groups. In case of type-A trichothecenes, the toxicity depends on the presence of an isovaleryl group and acetyl groups on the trichothecene core. Consequently, the cytotoxic potential of type-A trichothecenes is T-2 > HT-2 > Neosolaniol >T-2 triol > T-2 tetraol (Ueno, 1984). Primarily this cytotoxicity affects cell lines with a high division rates, like cells of the mucosa or the immune system (Bondy and Pestka, 2000). Thuvander et al. (1999) investigated the effect of trichothecenes on the human lymphocyte proliferation with *in vitro* studies. They showed that the type-A trichothecenes (T-2 and Diacetoxyscirpenol) supressed mitogen-induced lymphocyte proliferation 100 to 200 times more than type-B trichothecenes (Deoxynivalenol (DON) and Nivalenol (NIV)).

Moreover, T-2 was demonstrated to show the highest cytotoxic effect, which was already detectable at a half-maximal inhibitory concentration (IC_{50}) of 0.0182 µg/ml. In comparison the IC_{50} of NIV and ranged by 1.95 µg/ml and 16.93 µg/ml (Gareis, 2006).

2.3.5 Hazard characterisation and risk management

The above mentioned high prevalence of type-A trichothecenes in cereals and maize and their high toxic potential promoted the *Scientific Committee on Food* (SCF) of the European Union to investigate the occurrence of 12 different trichothecenes and their effects in humans for over 5 years (1998 - 2002), leading to several positions papers. In 2003 the SCF published the SCOOP task 3.2.10 laying down (temporary)-Tolerable Daily Intakes (t-TDIs) for the four trichothecenes DON, NIV, T-2 and HT-2, which have been reviewed and consolidated in 2004 (Schothorst and van Egmond, 2004).

A TDI refers to the estimated quantity of any given substance, which can be ingested daily without having any adverse health effects for the consumer. The TDI is used for the intake of substances, which were not intentionally added, such as food or feed contaminations. Therefore, even if potential harmful substance is ingested, a health risk is practically excluded, as long as the daily intake does not exceed the tolerable limit (BfR, 2020).

The TDI of 0.01 µg/kg for the sum of T-2 and HT-2 was set by the European Food Safety Authority in 2011 and re-evaluated in 2014 (EFSA, 2011, 2014). These TDI would have been exceeded by consumption of most of the samples investigated by (Gottschalk et al., 2009). Cereals such as oats, maize and wheat showed the highest levels of contamination. The subpopulation "children and infants" was particularly exposed. In order to monitor and subsequently reduce this exceedance the EU released a recommendation letter in 2013 on the presence of T-2 and HT 2 toxins in cereals and cereal products (European Union, 2013). According to this recommendation, oats should be further investigated if exceeding a concentration of 1000 µg/kg (sum of T-2 and HT-2) as unprocessed cereals, or 200 µg/kg (sum of T-2 and HT-2) in case of cereals or cereal products for humans.

2.3.6 Analytical Methods

Type-A trichothecenes can be analysed via various methods for qualitative and (semi-) quantitative determination. These methods are mainly based on the same procedure, consisting of sampling, extraction, clean-up, detection, quantification, and validation.

Regarding sampling, the EU laid down exact regulations for an appropriate sample procedure (European Union, 2006) containing e.g., weight or number of samples for oats or dry fruits, homogeneity and sample quality. Sample homogeneity may be achieved by the usual blending and grinding techniques (Crews et al., 2010). Extraction can be performed with polar solvents such as acetonitrile/water, methanol, chloroform, and ethanol with diluted acid or buffer at low pH (Crews, 2015). Different clean-up methods include solid phase extraction and immunoaffinity columns (Berthiller et al., 2017; Gottschalk et al., 2009; Pereira et al., 2015). For separation, various techniques are used such as gas chromatography (Schollenberger et al., 1998), thin layer chromatography (Sokolović and Šimpraga, 2006) or high performance liquid chromatography (HPLC) (Berthiller et al., 2017). Mass spectrometry (MS) is a reliable technique for the detection of type-A trichothecenes (Gottschalk et al., 2009; Radová et al., 1998; Schollenberger et al., 1998). The now preferred and commonly used determination is by (high performance) liquid chromatography-mass spectrometry.

Screening test for T-2 and HT-2 are used in routine analysis based on immunoassays (Li et al., 2014) or thin layer chromatography (Sokolović and Šimpraga, 2006).

In order to maintain sufficient and reliable performance characteristics, a method should be carefully validated. Method validation should include the limit of detection (LOD), limit of quantification (LOQ), (linear) working range, precision (repeatability and reproducibility), trueness (recovery), selectivity and robustness (Berthiller et al., 2017).

Since a LC-MS/MS method for type-A trichothecenes in edible insect material has not yet been established, a new method have been developed in the presented study. Homogenous, dry sample material was added with a 0.2 % formic acid /ACN (50/50, v/v) solution and extracted by ultrasonication and horizontal shaking adopted to Niermans et al. (2019). Clean-up was performed by adding anhydrous sodium sulphate and flowing centrifugation. Separation, identification, and quantification was conducted by HPLC coupled to MS. The method validation was conducted according to German standard norm DIN 32645 (2008-11). Detailed

method information is given in section “Materials and Methods” in Chapter 3. Figure 6 shows a typical chromatogram obtained after separation and detection by HPLC with MS of a mixture of T-2, HT-2, Triol and Tetraol with a concentration of 100ng/ml.

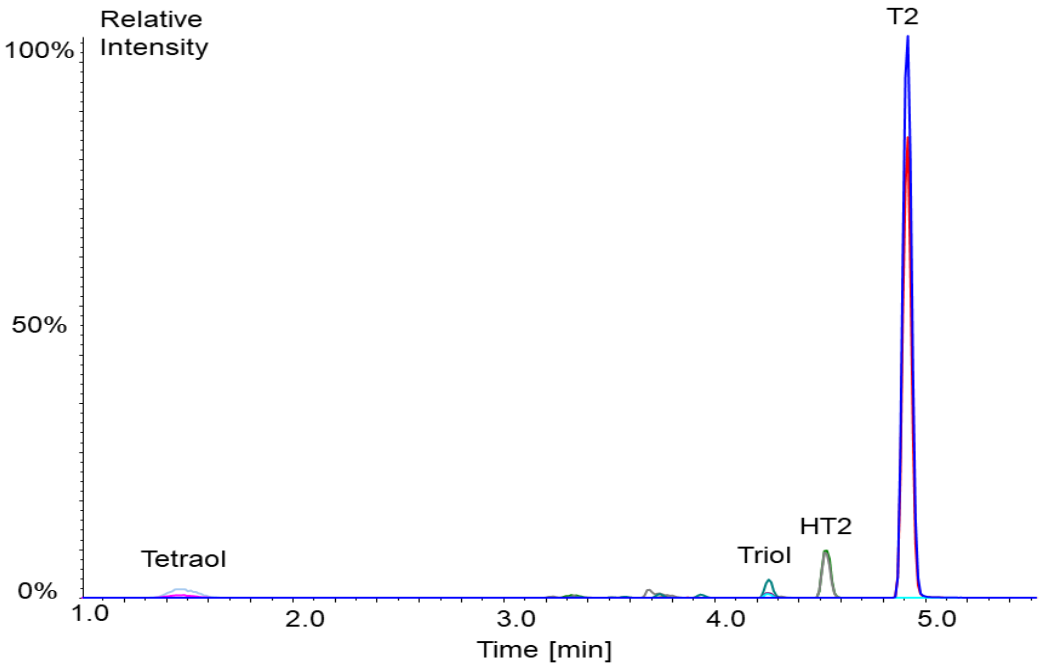

Figure 6 LC-MS/MS chromatogram of a standard mix solution (c= 100ng/ml) containing the 4 tested Trichothecenes, measured under Figure optimized chromatographic and mass spectrometric conditions.
(HT-2 = HT-2 toxin; T-2 = T-2 toxin; Tetraol = T-2 tetraol; Triol = T-2 triol)

3 Publication

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Distribution of T-2 toxin and HT-2 toxin during experimental feeding of yellow mealworm (*Tenebrio molitor*)

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List of abbreviations

Acetonitrile = ACN; artificial control = A(C); artificial low dose = A(LD); Artificial High Dose = A(HD); HT-2 toxin = HT-2; high performance liquid chromatography = HPLC; limit of detection = LOD; limit of quantification = LOQ; mass spectrometer = MS; natural control = N(C); natural low dose = N(LD); natural high dose = N(HD); T-2 toxin = T-2

Abstract

Within the European Union (EU), edible insects need to be approved as "Novel Food" according to Regulation (EU) 2015/2283 and must comply with the requirements of European food law with regard to microbiological and chemical food safety. Substrates used for feeding insects are susceptible to the growth of *Fusarium* spp. and consequently to contamination with trichothecene mycotoxins. Therefore, the current study aimed to investigate the influence of T-2 and HT-2 toxin on the larval life cycle of yellow mealworm (*Tenebrio molitor* (L.)) and to study the transfer of T-2, HT-2, T-2 triol and T-2 tetraol in the larvae. In a 4-week feeding study, *T. molitor* larvae were kept either on naturally (oat flakes moulded with *Fusarium sporotrichioides*) or artificially contaminated oat flakes, each at two levels (approximately 100 and 250 µg/kg total T-2 and HT-2). Weight gain and survival rates were monitored, and mycotoxins in the feeding substrates, larvae, and residues were determined using LC-MS/MS. Larval development varied between the diets and was 44 % higher for larvae fed artificially contaminated diets. However, the artificially contaminated diets had a 16 % lower survival rate. No trichothecenes were detected in the surviving larvae after harvest, but T-2 and HT-2 were found both in the dead larvae and in the residues of naturally and artificially contaminated diets.

Keywords

Yellow mealworm (*Tenebrio molitor*), edible insects, trichothecenes, food safety, mass spectrometry, biotransformation

Introduction

The fact that edible insects have been stated to be a potential source to reach the first three United Nations Sustainable Development Goals (no poverty, zero hunger, good health and well-being) (FAO 2009) has boosted scientific interest in not only the farming and processing of such animals but also consumer acceptance, nutritional value and safety aspects (Imathiu 2019). The economic value of insects has greatly increased, especially in Europe, and therefore ecological and economic perspectives are part of ongoing research (Kierończyk et al. 2019; Manzano-Agugliaro et al. 2012; Yen 2015).

Many of the more than 2100 insect species referred to as edible (Jongema 2017) seem to have an adequate profile of amino acids and fatty acids as well as mineral content to serve as a diet for vertebrates, e.g., as fish

feed or human nutrition (Barroso et al. 2014; Montowska et al. 2019; Yi et al. 2013). Additionally, the high protein content of insects, ranging from 52 to 76 % (Zielińska et al. 2015), is seen as one of the major benefits for a future human and animal diet. Especially in Europe, concerns about the safety of insect products are leading to refusal of such products or even disgust (Bednářová et al. 2013; Hartmann et al. 2015; Yen 2009). To dissipate these concerns and develop safety schemes for farming and processing, various aspects, such as microbiology (Klunder et al. 2012), pesticides and heavy metals (Poma et al. 2017), have been investigated and addressed in European guidelines (IPIFF 2019).

Recent studies have shown that insects – if kept on rotten and/or mouldy substrates – may render hazardous metabolites harmless via their own metabolism or largely excrete them, especially mycotoxins, such as zearalenone or type B trichothecenes (Niermans et al. 2019; Ochoa Sanabria et al. 2019; Van Broekhoven et al. 2017). However, as shown for zearalenone, metabolism in *T. molitor* may also result in the formation of compounds of higher toxicity, such as α-zearalenol (Niermans et al. 2019). Based on these studies, it could be possible to obtain a safe product for further use for food or feed through insects reared on material no longer suitable for animal or human consumption. However, as described for aflatoxin B1 (Bosch et al. 2017) or type B trichothecenes and ochratoxins (Camenzuli et al. 2018), the various insect species appear to have different metabolization and excretion pathways for mycotoxins. However, comprehensive investigations of the metabolism and fate of trichothecenes, especially type A trichothecenes in *T. molitor*, are so far not available. In general, type A trichothecenes show several adverse effects, e.g., cytotoxicity and leukopenia in mammals (Bauer et al. 1989; Li et al. 2011), and can be found in various grain samples (EFSA 2017). Additionally, livestock farming is negatively affected by trichothecene-contaminated feed, e.g., for pigs, poultry, and horses (EFSA 2011). Therefore, edible insects could be a high-value and safe food or feed alternative if trichothecenes do not accumulate, especially as the utilization of grains containing high amounts of mycotoxin of natural origin has been shown to positively affect the growth rate of *T. molitor* (Niermans et al. 2019).

The aims of the present study were to investigate the effects on the weight gain and survival of *T. molitor* larvae fed different diets containing two amounts of T-2 and HT-2 toxins. The feed was either naturally contaminated by the addition of *F. sporotrichioides*-contaminated oat flakes or artificially contaminated with T-2/HT-2 standards. After a four-week feeding period, the occurrence of T-2, HT-2, T-2 triol and T-2 tetraol in the larvae and their residues was determined and evaluated. This study aimed to assess possible degradation pathways of type A trichothecenes in *T. molitor* larvae and to examine the ability of these species to be safe utilizers of trichothecene-contaminated diets.

Materials and Methods

Chemical reagents and standards

The following mycotoxin calibrant solutions (Biopure™ certified reference materials, purity (HPLC) > 98.9 %) were purchased from Romer Labs (Getzersdorf, Austria) and were used for all experiments and measurements. The solutions were used within the indicated expiry date: T-2 toxin (T-2, c = 101.2 µg/mL), HT-2 toxin (HT-2, c = 100.1 µg/mL), T-2 triol (Triol, c = 50.1 µg/mL) and T-2 tetraol (Tetraol, c = 50.1 µg/mL). Acetonitrile (ACN, LC-MS grade) and formic acid (p.a. grade) were purchased from Th. Geyer (Renningen, Germany) and were used for all experiments and analyses. A standard mixed solution containing all four analytical standards (c = 1.0 µg/mL) was prepared with ACN and stored at 6 °C in the dark. Ultrapure water was obtained using an UltraClear TM TP UV UF TM system from Evoqua Water Technologies (Barsbüttel, Germany) and was used for all experiments and analyses unless otherwise stated. Sodium sulphate (anhydrous) was purchased from Merck (Darmstadt, Germany). Ammonium formate was obtained from Fluka (Steinheim, Germany).

Feeding substrates and diet preparation

Two different kinds of diets with two mycotoxin levels each were prepared for the feeding experiment. Diet “A” consisted of oat flakes that were artificially contaminated with T-2 and HT-2 toxin. Diet “N” was prepared by using naturally contaminated oat flakes containing a toxigenic *Fusarium* strain of mould (see below). The oat flakes used in these experiments were purchased from a local supermarket and were intended for human consumption. The levels of contamination with T-2 and HT-2 were chosen in reference to the European Commission "indicative levels for unprocessed cereals" as published by the European Commission recommendation 2013/165/EU for compound feed (EC, 2013). Therefore, two diets with toxin levels of approximately 100 µg/kg (low dose) and 250 µg/kg (high dose) (sum of T-2 and HT-2 toxin) were prepared as shown in Table 1.

For preparation of the artificially contaminated diets, oat flakes were milled to flour (< 0.5 mm particle size) using a Grindomix 200 centrifugal mill (Retsch, Haan, Germany) and were contaminated with T-2 and HT-2 to obtain the diets A(LD) and A(HD). For that purpose, standards of T-2 and HT-2 were added to 200 g of oat flakes soaked in 1 L of distilled water, i.e., 10 µg of each toxin for the lower dose A(LD) and 25 µg for the higher dose A(HD). The preparations were mixed vigorously for 1 h in a 2 L Erlenmeyer flask by a magnetic stirrer and afterwards dried at 50 °C in a drying cabinet (FD-115, Binder, Tuttlingen, Germany) for 4 h and

lyophilised (CTFD-10P, Berrytec, Grünwald, Germany) to a stable weight for 72 h. After lyophilisation, the samples were again milled to a particle size of < 0.5 mm. For preparation of the uncontaminated control diet A (C), oat flakes were treated identically, excluding the addition of mycotoxin standards.

For preparation of the naturally contaminated diets, oat flakes (50 g) were autoclaved (121.1 °C, 15 min) with 100 mL of distilled water in an Erlenmeyer flask. Afterwards, a spore suspension of *F. sporotrichioides* var. *minus* Wollenweber 1930 (strain DSM No. 62425, obtained from the German Collection of Microorganisms and Cell Cultures – DSMZ, Braunschweig, Germany) was added. For this purpose, the strain was cultivated on malt-extract agar for three weeks, and spores were washed off with 2 mL of distilled water. After two weeks of incubation at 25 °C, the mouldy material was autoclaved again, dried and milled to flour (< 0.5 mm particle size). The naturally contaminated material contained 260 mg/kg T-2 and 18.2 mg/kg HT-2 as measured by LC-MS/MS (method described below). As a consequence of this natural co-occurrence, the same T-2/HT-2 ratio was also present in the prepared diets. Due to the high toxin amount in the moulded oat flakes, 5.0 g of the material was pre-diluted 1:40 (195 g blank milled oat flakes) and homogenised in a 500 mL polyethylene vessel by using a Reax 2 overhead shaker (Heidolph, Schwabach, Germany) for 4 h. For the diets N(LD) and N(HD), 3.2 g and 8.0 g of diluted moulded oat flakes were mixed with blank milled oat flakes to a total of 200 g and homogenised again as described above. Uncontaminated milled oat flakes were used as the control group N(C). To determine the homogeneity of the produced diets, ten samples were randomly taken from each contaminated diet and analysed according to the LC-MS/MS protocol as given below. The results revealed relative standard deviations ranging from 10.0 % for N(LD) to 19.6 % for N(HD). The homogeneity was therefore considered satisfactory. The results of the controls were all < LOD for all four measured type A trichothecenes.

For quality control, the total energy and protein content in the feeding substrates were monitored (see Table 1). The total energy of the diets was determined as the heat of combustion by adiabatic bomb calorimetry (2 repetitions, IKA C2000, Rhys International Ltd, Bolton, UK) and ranged from 17.8 to 18.7 MJ/kg. For the determination of dry matter, the samples were dried at 103 °C until stable weight and weighed. The protein content was determined by the method of Dumas in a LECO FP-248 Model Nitrogen Determinator (Leco, Mönchengladbach, Germany) and ranged from 14.3 to 15.8 % on a dry matter basis (Table 1).

Selection, exposure, and harvest of larvae

T. molitor larvae, kindly provided by the Institute for Food Technology and Biochemical Engineering of Bremerhaven University of Applied Sciences, Germany, were initially kept on wheat bran as substrate and were

selected at an age of 42 days with a size of approximately 1 cm. Species identification was conducted via PCR in our laboratory (unpublished method). Before starting the feeding experiment, the larvae were starved for 48 h and divided into 16 diet groups, each containing 200 individuals with an average weight of 8.8 ± 0.3 mg per individual. The larvae were kept in 400 mL polyethylene cups for an exposure time of 4 weeks at 28 °C and 80 % humidity with a 12 h day and night light rhythm. Each group was fed *ad libitum* with a total of 6 g of the designated diet. Experiments with the toxin-containing diets were performed in biological triplicate and blank control diets in duplicate (see. Figure 1). During the experiment, the biological parameters larval weight gain and survival rate, measured as the total amount of dead larvae for each diet, were recorded weekly. Larvae that died during the 4-week feeding experiment were separated from the living larvae and stored at -18 °C. At the end of the exposure time, the larvae were harvested and stored – along with the residues (mixture of moults, faeces, and remaining feed in the cups) – at -18 °C before lyophilisation for 72 h.

Sample preparation of oat feed

The sample preparation procedure was based on Niermans et al. (2019) with slight modifications. Homogenous, dry oat flakes (2.0 g) were weighed in a 50 mL polystyrene tube, and 15 mL of 0.2 % formic acid/ACN (50/50, v/v) was added. The samples were extracted by ultrasonication (10 min) and horizontally shaken (500 rpm, 30 min). After centrifugation for 10 min (4100×*g*, 10 °C), 1 mL of supernatant was transferred into a 2 mL reaction tube, and 250 mg of anhydrous sodium sulphate was added to separate the organic and water phases. After mixing for 30 s with a vortex laboratory shaker, the samples were again centrifuged (12800 ×g, 10 °C, 20 min). An aliquot of the supernatant (200 µL) was diluted with 1800 µL of water and filtered into an HPLC-glass vial using a 0.2 µm RC syringe filter (Berrytec, Grünwald, Germany).

Sample preparation of larvae/residues

Lyophilised larval samples were ground with a mortar and pestle, and 200 mg of dry sample material was weighed in duplicate into a 2 mL reaction tube. Samples were extracted with 1.5 mL of 0.2 % formic acid/ACN (50/50, v/v) in an ultrasonic bath (10 min) and horizontally shaken (500 rpm, 30 min). After centrifugation for 10 min (see above), samples were further treated as described before.

LC-MS/MS instrumentation

A Shimadzu high-performance liquid chromatography (HPLC) apparatus including binary pumps, a degasser, an autosampler, a column oven and a control unit (LC-20AB, SIL-20AC HT, CTO-20AC, CBM-20A, Duisburg,

Germany) was used for all measurements. The HPLC was coupled to an API4000 triple quadrupole mass spectrometer (MS) provided by Sciex (Darmstadt, Germany). The MS ion source parameters were set as follows: ESI + ionization voltage, 4.200 V; nebulizer gas, 50 psi; heating gas, 50 psi; curtain gas, 35 psi; temperature, 550 °C; collision gas level, 7. The MS parameters used are summarized in Online Resource 1. Data acquisition and processing were conducted with Sciex Analyst (Version 1.6.2) and MultiQuant software (Version 3.0.1).

Measurements and quantification

For chromatographic separation of T-2, HT-2, T-2 triol and T-2 tetraol, a 50 x 2.1 mm Kinetex™ 2.6 µm CoreShell EVO C18 100 Å column protected by a SecurityGuard™ ULTRA EVO C18 2.1 mm guard column (both Phenomenex, Aschaffenburg, Germany) was used. HPLC solvents were water and acetonitrile/water (95/5, v/v, B), each containing 0.1 % formic acid and 5 mmol/L ammonium formate. The column oven temperature was maintained at 30 °C, and 20 µL of sample extract was injected. The binary linear gradient conditions at a flow rate of 0.4 mL/min were 0.0 min 2 % B, 5.5 min 100 % B, 8.5 min 100 % B and additional re-equilibration of 2.2 min prior to each run. The quantification was performed via external matrix matched calibration (linear regression). Standards were freshly prepared on each day of measurement. Aliquots of the mixed standard solution containing T-2, HT-2, T-2 triol and T-2 tetraol were pipetted into glass vials, dried under a gentle flow of nitrogen at 50 °C and reconstituted with extracts of blank oat flakes (prepared as described above) and larvae/residues obtained from the blank diets (N(C), A(C)) to compensate for matrix effects. The concentrations of the calibration standards were 0, 1.0, 2.5, 5.0, 10, 25, 50 and 100 ng/mL.
Statistical analyses of the processed data were conducted in Excel 2016 and R (Version 3.6.2, R Core Team 2019). A linear regression model was used to test the data for significance and plausibility. Figures were drawn using OriginPro software (Version 2020, OriginLab, Northampton, USA).

Method performance and validation

Recovery rates were assessed for larvae, oat flakes, and residue samples after artificial contamination of each matrix with the four analytes at two levels (75 µg/kg and 187.5 µg/kg). T-2 triol recovery in the residues was assessed by artificial contamination at the levels of 187.5 µg/kg and 300 µg/kg. Limits of detection (LOD), limits of quantification (LOQ) and linearity were calculated according to the calibration curve method of German standard norm DIN 32645 (Chemical analysis –Decision limit, detection limit and determination limit under repeatability conditions –Terms, methods, evaluation- (2008-11)). Intraday precision (RSD_r) was determined as the relative standard deviation of six replicates for each sample material and toxin amount. The

interday precision (RSD_R) was determined by the preparation and measurement of one sample on five consecutive days (see Table 2). Amounts smaller than the LOD were treated as "0 µg/kg". Levels < LOQ were treated as 0.5 LOQ. All results were related to dry matter and not corrected for recovery rates.

Results

Method performance

LODs between 2.1 µg/kg for T-2 and 69.0 µg/kg for T-2 triol (each in residues) were calculated for each single matrix (oat flakes, larvae, residues). The LOQs ranged from 7.0 µg/kg for T-2 to 184.4 µg/kg for T-2 triol (Table 2). Recovery rates (% ± RSDr) after artificial contamination of all sample materials with the four analytes ranged from 50.1 ± 22.8 % for T-2 tetraol (in residue and larvae) to 116.1 ± 16.3 % for T-2 triol (in residue) at the low level of fortification and from 48.5 ± 9.5 % for T-2 tetraol (in larvae) to 134.3 ± 7.2 % for HT-2 triol (in larvae) at the high level of fortification (see Table 2). For interday precision, RSD_R values from 6.9 % for T-2 in larvae to 25.4 % for T-2 triol in oat flakes (low level) were calculated. For the high level, RSD_R ranged from 1.9 % for T-2 in residue to 27.1 % for T-2 triol in oat flakes.

Biological parameters

During the 4 weeks of exposure, the 6 feeding groups differed in their development. The larval weight gain of the artificial diets, including the control group A(C), was significantly ($P < 0.001$) higher than that of the naturally contaminated diets; e.g., larvae fed the A(HD) diet gained 44.1 ± 3 % (average ± RSD) more weight than the N(C) groups (see Figure 2 A). In total, their weight increased by 113.8 ± 1 % compared to an average weight gain of 92.8 ± 21 % over all diets. Moreover, larval growth differed significantly ($P < 0.05$) between diets with different toxin levels. Compared to the percentage weight gain (comparing start and end of the experiment) in the control diet (N(C)) groups, the groups fed both contaminated diets gained between 2.1 – 17.6 % (N(LD)) and 7.6 to 11.0 % (N(HD)) more weight. The A(LD) diet groups gained between 11.2 and 21.5 % more weight than A(C) (see Table 3). The additional weight gain of the A(HD) groups ranged from 17.4 to 19.8 %. Overall, larval growth was highest during the first week: larvae of all diets showed 51 ± 23 % weight gain in this period, which then decreased steadily from week to week until the growth gain was 4.4 ± 36 % from week 3 to week 4 (see. Figure 2 A).

After 4 weeks of exposure, larval death was observed in all blank and contaminated diets, except for one replicate of the control group N(C), in which all 200 individuals survived (Table 3). On average, the survival rate was 88.6 ± 10 % (see Table 3). Compared to the control group N(C) (97.3 ± 4.0 %), the survival of N(LD) (96.0 %) and N(HD) (97.2 %) was reduced or equal, with high biological variation, similar to that in the groups fed the artificially contaminated diets (see. Figure 2 B). However, a highly significant difference ($P < 0.001$) was observed between the natural and artificial diets (including the blank groups in both diets), resulting in an average decrease of 16.2 % in the survival rate of the larvae fed the artificial diets (see Table 3).

Occurrence of T-2 and metabolites in larvae and residues

Larvae and residues of each diet group were tested for the presence of selected type A trichothecenes. Among the samples of surviving larvae, neither T-2 nor any of its metabolites (HT-2, T-2 triol, T-2 tetraol) was detected. One pooled sample of the larvae that died during 4 weeks of exposure (dead larvae) was measured to achieve sufficient sample material for each diet group, as the cumulated dry weight of the dead larvae (72 ± 16 mg) was insufficient for assessing singular replicates.

None of the four selected trichothecenes were detected in the dead larvae of the control diet groups N(C) and A(C). HT-2, T-2 tetraol and T-2 triol were not detected in any of the dead larvae samples, but the dead larvae from the N(HD)-group contained up to 44.2 µg/kg of T-2. Lower amounts of T-2 toxin (7.7 µg/kg, > LOD - < LOQ) were found in the larvae of the diet groups N(LD), A(LD), and A(HD) (see Table 4). The control diets N(C) and A(C) were free from the investigated type A trichothecenes.

It was not possible to investigate the larval residue separately, and neither qualitative results nor feed conversion could be derived, as the milled oat flakes had been further minced by the larvae during the feeding period, resulting in a mixture of oat flakes and larval residue at the end of the 4-week exposure time. However, compared to the fed contaminated oat diets, there was a reduction of T-2/HT-2 toxin levels in the residues (Table 4).

Discussion

Our data revealed that the intake of type T-2 and HT-2 toxins, at total amounts of approximately 100 and 250 µg/kg, had a significant influence on the weight gain and survival of *T. molitor* larvae compared to the control groups. Additionally, the preparation method of the contaminated oat flakes affected the biological parameters, not only leading to increased body weight in the artificial diet groups (fortification of oat flake slurry

with pure standard) but also to increased mortality of these larvae. With the current scientific knowledge, some of the observed effects cannot be definitely explained.

Regarding the biological parameters, Van Broekhoven et al. (2014) observed increased weight gain in *T. molitor* larvae fed T-2-contaminated diets with an almost unaffected survival rate (98 %). Additionally, the results of Davis and Schieffer (1982) indicated that larval survival was not influenced by the amount of T-2, but the weight gain decreased with increasing levels of the toxin. Type B trichothecenes such as deoxynivalenol (DON) can lead to reduced larval body weight, locomotor activity and protein content (Janković-Tomanić et al. 2019). However, not only single mycotoxins can be regarded as the sole factors influencing larval biological parameters, especially since *Fusarium* spp. are able to form a variety of different toxic metabolites. For example, *F. sporotrichioides* is capable of producing at least 17 toxic metabolites (Thrane et al. 2004). The observations of larval biological parameters in the naturally contaminated diets could be attributed to the sum of existing mycotoxins, i.e., not only to the four analysed mycotoxins but also to metabolites that have not been further investigated in the present study. Guo et al. (2014) showed that the consumption of some *Fusarium* spp. can lead to elevated mortality rates and that *T. molitor* larvae showed feed preferences resulting in avoidance of contaminated diets with potential survival threats caused by some *Fusarium* species. However, *Fusarium graminearum*-contaminated diets positively affected larval weight gain (Niermans et al. 2019), as was also observed with the *F. sporotrichioides*-contaminated diets in this feeding study. A corresponding observation from an earlier study was attributed to the fungus or other beneficial fungal secondary products (van Broekhoven et al. 2014). However, as the total energy and dietary protein can be considered equal for the different diet groups in the present feeding trial (see Table 1), the effects on larvae seemed to be due to the toxins or the different preparation methods for the diets.

T-2 is known to inhibit protein synthesis and induce apoptosis (Shifrin 1999) in mammalian cells, e.g., in reproductive, gastrointestinal and dorsal skin tissue (Doi et al. 2006). Therefore, if T-2 is assumed to have similar effects in insect cells, the intake of trichothecenes could lead to a reduced growth rate or an increased mortality of mealworm larvae. However, biotransformation pathways for mycotoxins, in *T. molitor* or any other insect, have not yet been described, complicating the interpretation. In comparison, the T-2 degradation pathway in mammals can be divided into two phases (Bauer et al. 1989; Ueno 1984). T-2 is mainly metabolized in the intestinal epithelium and the liver and subsequently conjugated and detoxified (Bauer et al. 1989; Conrady-Lorck et al. 1988). Excretion in mammals occurs via the faeces and urine (Pfeiffer et al. 1988). As this class of insects, in particular the beetle family Tenebrionidae, has different anatomy and physiology from mammals, it is

assumed that the degradation pathway of trichothecenes is also different, in particular because insects are able to detoxify natural and synthetic noxious agents in a way that is impossible for mammals (Bass et al. 2015; Ivie et al. 1983).

Although several bacteria communities are capable of metabolising T-2 (Dohnal et al. 2008; Wachowska et al. 2017) and can be abundant in the *T. molitor* microbiome (Garofalo et al. 2019), their potential to metabolize all incidental toxins is unknown. Excretion of mycotoxins appears to occur through the larval faeces (Niermans et al. 2019; Ochoa Sanabria et al. 2019; Van Broekhoven et al. 2017). Therefore, a symbiosis of bacterial communities and larval metabolism could be possible and could lead to an unknown pathway, resulting in modification or putative degradation of T-2/HT-2 toxins in the larvae.

Another aspect that must be considered regarding larval weight gain and survival is differences in diet preparation. As the dry matter of naturally and artificially contaminated diets differed by up to 5 %, it is conceivable that the larvae fed artificial diets had a higher nutrient intake due to higher dry matter and consequently higher energy density, leading to more weight gain, as was observed during the feeding experiment. Here, the larvae of A(C) gained significantly more weight than the larvae of N(C). However, as the oat flakes were prepared as a slurry in distilled water and heat treated during artificial diet preparation, it is possible that through these preparation methods, vitamins or other sensitive micronutrients were degraded or their content at least diminished. Because of the high metabolism of mealworm larvae and their repeated moulting processes, vitamin malnutrition is possible in a short time span and could lead to the increased mortality of the A(C) larvae compared to the N(C) larvae. The two diets also differed in terms of their T-2:HT-2 ratio. It cannot be excluded that these differing ratios could also have contributed to the observed differences. Consequently, diet preparation should always be considered as an influencing factor in such feeding experiments.

Regarding the detection of mycotoxins, none of the four investigated trichothecenes were detectable in the living larvae. Therefore, it seems apparent that both toxins present in the diets were transformed into unknown metabolites or degraded. In the latter case, the larvae – when harvested as fit, living and authentic organisms for food production according to the general food law (EC 2002) – can be regarded as a potential degrader of T-2/HT-2 from contaminated diets. These findings are in line with the results of Van Broekhoven et al. (2017), who conducted a feeding experiment with *T. molitor* larvae grown on wheat flour naturally contaminated with mycotoxins (inter alia, the structurally similar type B trichothecene deoxynivalenol). According to their data, deoxynivalenol was not detected in the harvested larvae but was detected in larval faeces. Additionally, several other trichothecene-contaminated feeding trials have been conducted with *T. molitor* larvae (Davis and Schieffer

1982; Niermans et al. 2019; Ochoa Sanabria et al. 2019), and all pointed towards degradation of mycotoxins by the larvae. However, if unknown metabolites are formed, detoxification in the living larvae cannot be assumed as long as these compounds are not identified. To evaluate possible remaining cytotoxicity from unknown metabolites, nontargeted studies should be performed with a bioassay such as the MTT cell culture assay (Gareis 2006; Hanelt et al. 1994). Furthermore, putative chemical modifications of the toxins, e.g., adduct formation with glucuronic acid or sulphates, could be studied by incubation with the respective enzymes. The usage of radiolabelled toxins could lead to further clarification of metabolic processes as well.

Potential cross contamination could be the reason for the exclusive detection of T-2 in dead larvae, e.g., through contaminated oat particles on the larval exoskeleton or in the digestive tract. However, this appears unlikely, as no T-2/HT-2 was detected in the identically treated surviving larvae. On the other hand, it seems more likely that the biotransformation pathway of the toxins was interrupted in the dead larvae. As the sample volume of dead larvae was low and samples had to be pooled, these observations should be reassessed in further studies. A higher number of individual larvae in each biological replicate, higher toxin levels and the assessment of the influence of extrinsic factors, such as temperature and humidity, on larval metabolism would also be of high interest. As discussed before, the putative formation of unknown metabolites should also be studied to better understand the biotransformation and impact of type A trichothecenes on mealworm larvae.

When evaluating the results of the residue samples, the reduction of both T-2 and HT-2 in these samples strongly pointed towards unknown metabolic processes since the trichothecene levels of the diets can be seen as stable during the feeding trial (Omurtag 2008). If T-2 triol was excreted in low amounts during the feeding experiment, a false negative result also appears to be possible, as the validated method is quite insensitive regarding the LOD detection level in residue. Furthermore, a separate analysis of residue alone was not possible. The T-2 levels detected in dead larvae as well as T-2 and HT-2 levels in residues are – compared to the regulatory limits on toxin levels in cereals in 2013/165/EU (EC 2013) – quite low. As neither dead or perished animals nor excrements are allowed to be used or processed as food in the European Union (EC 2002), these results are interesting from a scientific point of view but have no impact on the safety of insects for food use, provided that current food legislation is respected.

In conclusion, *T. molitor* larvae were affected by the trichothecenes as well as by the diet preparation itself. Larval growth was positively influenced by the addition of trichothecene-containing *F. sporotrichioides*, but the toxin amount had no effect on larval survival within the two diet groups. The detection of T-2 in dead but not in living larvae indicated a yet unknown metabolic or biotransformation process in the larvae. Therefore, the results

indicated that *T. molitor* larvae (harvested as healthy, living organisms) are potential degraders of T-2/HT-2 toxins. However, further studies on biotransformation pathways and the effects of potentially formed metabolites are still needed.

Conflicts of interest: None.

References

Barroso FG, de Haro C, Sánchez-Muros MJ, Venegas E, Martínez-Sánchez A, Pérez-Bañón C (2014) The potential of various insect species for use as food for fish. Aquaculture 422-423:193-201. https://doi.org/10.1016/j.aquaculture.2013.12.024

Bass C, Denholm I, Williamson MS, Nauen R (2015) The global status of insect resistance to neonicotinoid insecticides. Pestic Biochem Physiol 121:78-87. https://doi.org/10.1016/j.pestbp.2015.04.004

Bauer J, Gareis M, Gedek B (1989) Metabolism of the trichothecenes T-2 toxin, diacetoxyscirpenol, and deoxynivalenol by farm animals. In: Chełkowski J (ed) Topics in Secondary Metabolism, vol 2, Fusarium: Mycotoxins, Taxonomy, Pathogenicity. Elsevier, New York, pp 139-165. https://doi.org/10.1016/B978-0-444-87468-9.50013-4

Bednářová M, Borkovcová M, Mlček J, Rop O, Zeman L (2013) Edible insects - species suitable for entomophagy under condition of Czech Republic. Acta Univ Agric Silvic Mendelianae Brun 61:587-593. https://doi.org/10.11118/actaun201361030587

Bosch G, Fels-Klerx HJV, Rijk TC, Oonincx D (2017) Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (Hermetia illucens) and yellow mealworms (Tenebrio molitor). Toxins (Basel) 9:185. https://doi.org/10.3390/toxins9060185

Camenzuli L, van Dam R, de Rijk T, Andriessen R, van Schelt J, van der Fels-Klerx HJI (2018) Tolerance and excretion of the mycotoxins aflatoxin B(1), zearalenone, deoxynivalenol, and ochratoxin a by Alphitobius diaperinus and Hermetia illucens from contaminated substrates. Toxins 10:91. https://doi.org/10.3390/toxins10020091

Conrady-Lorck S, Gareis M, Feng XC, Amselgruber W, Forth W, Fichtl B (1988) Metabolism of T-2 toxin in vascularly autoperfused jejunal loops of rats. Toxicol appl pharmacol 94:23-33. https://doi.org/10.1016/0041-008X(88)90333-X

Davis GRF, Schieffer HB (1982) Effects of dietary T-2 Toxin concentrations fed to larvae of the yellow mealworm at three dietary protein levels. Comp Biochem Physiol 73: 13-16. https://doi.org/10.1016/0306-4492(82)90159-9

Dohnal V, Jezkova A, Jun D, Kuca K (2008) Metabolic pathways of T-2 toxin. Curr drug metab 9:77-82. https://doi.org/10.2174/138920008783331176

Doi K, Shinozuka J, Sehata S (2006) T-2 toxin and apoptosis. Toxicol Pathol 19:15-27. https://doi.org/10.1293/tox.19.15

EC- European Commission (2002) Commission regulation (EC) No 178/2002 of the European parliament and of the council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European food safety authority and laying down procedures in matters of food safety. Off J Eur Comm L 031: 1–24. Last consolidated version available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX:32002R0178

EC- European Commission (2013) Commission recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. Off J Eur Comm L 91: 12–15. Last consolidated version available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32013H0165

EFSA - European Food Safety Authority, Panel on Contaminants in the Food Chain (2011) Scientific opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J 9(12):2481-2668. https://doi:10.2903/j.efsa.2011.2481

EFSA - European Food Safety Authority (2017) Scientific report on human and animal dietary exposure to T-2 and HT-2 toxin. EFSA J 15(8):4972- 129. https://doi.org/10.2903/j.efsa.2017.4972

EU - European Union (2015) Commission regulation (EU) 2015/2283 of the European parliament and of the council of 25 November 2015 on novel foods, amending regulation (EU) No 1169/2011 of the European parliament and of the council and repealing regulation (EC) No 258/97 of the European parliament and of the council and Commission regulation (EC) No 1852/2001. Off J Eur Comm L 327: 1–22 Last consolidated version available from: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32015R2283

FAO - Food and Agriculture Organization of the United Nations (2009) How to feed the world in 2050. https:// www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf. Accessed 25 August 2020

Gareis M (2006) Diagnostischer Zellkulturtest (MTT-Test) für den Nachweis von zytotoxischen Kontaminanten und Rückständen. J Consumer Prot Food Saf, 1, 354-363. https://doi.org/10.1007/s00003-006-0058-6

Garofalo C, Milanović V, Cardinali F, Aquilanti L, Clementi F, Osimani A (2019) Current knowledge on the microbiota of edible insects intended for human consumption: a state-of-the-art review. Food Res Int: 108527. https://doi.org/10.1016/j.foodres.2019.108527

Guo Z, Doll K, Dastjerdi R, Karlovsky P, Dehne HW, Altincicek B (2014) Effect of fungal colonization of wheat grains with fusarium spp. on food choice, weight gain and mortality of meal beetle larvae (Tenebrio molitor). PLOS ONE https://doi.org/10.1371/journal.pone.0100112

Hanelt M, Gareis M, Kollarczik B (1994) Cytotoxicity of mycotoxins evaluated by the MTT-cell culture assay. Mycopathologia 128:167-174. https://doi.org/10.1007/BF01138479

Hartmann C, Shi J, Giusto A, Siegrist M (2015) The psychology of eating insects: a cross-cultural comparison between Germany and China. Food Qual Preference 44:148-156. https://doi.org/10.1016/j.foodqual.2015.04.013

Imathiu S (2019) Benefits and food safety concerns associated with consumption of edible insects. NFS J 18:1-11. https://doi.org/10.1016/j.nfs.2019.11.002

IPIFF- International Platform of Insects for Food and Feed (2019) Guidance the provision of food information to consumers edible insect-based products. https://ipiff.org/wp-content/uploads/2019/12/IPIFF-Guide-on-Good-Hygiene-Practices.pdf. Accessed 19 February 2020

Ivie GW, Bull DL, Beier RC, Pryor NW, Oertli EH (1983) Metabolic detoxification: mechanism of insect resistance to plant psoralens. Science 221:374-376. https://doi.org/10.1126/science.221.4608.374

Janković-Tomanić M, Petković B, Todorović D, Vranković J, Perić-Mataruga V (2019) Physiological and behavioral effects of the mycotoxin deoxynivalenol in Tenebrio molitor larvae. J Stored Prod Res 83:236-242. https://doi.org/10.1016/j.jspr.2019.07.008

Jongema Y (2017) Worldwide list of recorded edible insects. Wageningen University & Research, https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.html. Accessed 19 Februrary 2020

Kierończyk B, Sypniewski J, Rawski M, Czekała W, Świątkiewicz S, Józefiak D (2019) From waste to sustainable feed material: the effect of Hermetia illucens oil on the growth performance, nutrient digestibility, and gastrointestinal tract morphometry of broiler chickens. Ann Anim Sci 20:157-177. https://doi.org/10.2478/aoas-2019-0066

Klunder HC, Wolkers-Rooijackers J, Korpela JM, Nout MJR (2012) Microbiological aspects of processing and storage of edible insects. Food Control 26:628-631. https://doi.org/10.1016/j.foodcont.2012.02.013

Li Y, Wang Z, Beier RC, Shen J, De Smet D, De Saeger S, Zhang S (2011) T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods. J Agric Food Chem 59:3441-3453. https://doi.org/10.1021/jf200767q

Manzano-Agugliaro F, Sanchez-Muros MJ, Barroso FG, Martínez-Sánchez A, Rojo S, Pérez-Bañón C (2012) Insects for biodiesel production. Renew Sustain Energy Rev 16:3744-3753. https://doi.org/10.1016/j.rser.2012.03.017

Montowska M, Kowalczewski PL, Rybicka I, Fornal E (2019) Nutritional value, protein, and peptide composition of edible cricket powders. Food Chem 289:130-138. https://doi.org/10.1016/j.foodchem.2019.03.062

Niermans K, Woyzichovski J, Kroencke N, Benning R, Maul R (2019) Feeding study for the mycotoxin zearalenone in yellow mealworm (Tenebrio molitor) larvae-investigation of biological impact and metabolic conversion. Mycotoxin Res 35:231–242. https://doi.org/10.1007/s12550-019-00346-y

Ochoa Sanabria C et al. (2019) Yellow mealworm larvae (Tenebrio molitor) fed mycotoxiN(C)ontaminated wheat-a possible safe, sustainable protein source for animal feed? Toxins (Basel) 11(5):282. https://doi.org/10.3390/toxins11050282

Omurtag GZ (2008) Fumonisins, trichothecenes and zearalenone in cereals. Int J Mol Sci 9:2062-2090. https://doi.org/10.3390/ijms9112062

Pfeiffer RL, Swanson SP, Buck WB (1988) Metabolism of T-2 toxin in rats: effects of dose, route, and time. J Agric Food Chem 36:1227-1232. https://doi.org/10.1021/jf00084a025

Poma G, Cuykx M, Amato E, Calaprice C, Focant JF, Covaci A (2017) Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. Food Chem Toxicol 100:70-79. https://doi.org/10.1016/j.fct.2016.12.006

Shifrin V, Anderson P (1999) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase and induces apoptosis. J Biol Chem 274:13985-13992. https://doi.org/10.1074/jbc.274.20.13985

Thrane U, Adler A, Clasen PE et al. (2004) Diversity in metabolite production by Fusarium langsethiae, Fusarium poae, and Fusarium sporotrichioides. Int J Food Microbiol 95:257-266. https://doi.org/10.1016/j.ijfoodmicro.2003.12.005

Ueno Y (1984) Toxicological features of T-2 toxin and related trichothecenes. Fundam appl Toxicol 4:124-132. https://doi.org/10.1093/toxsci/4.2parT-2.124

van Broekhoven S, Dohan Q, van Huis A, van Loon JJA (2014) Exposure of tenebrionid beetle larvae to mycotoxiN(C)ontaminated diets and methods to reduce toxin levels. Netherlands Entomological Society Meeting; FAO, Rome, Italy

van Broekhoven S, Gutierrez J, De Rijk TC, De Nijs WCM, van Loon JJA (2017) Degradation and excretion of the Fusarium toxin deoxynivalenol by an edible insect, the yellow mealworm (Tenebrio molitor L.). World Mycotoxin J 10:163-169. https://doi.org/10.3920/wmj2016.2102

Wachowska U, Packa D, Wiwart M (2017) Microbial inhibition of Fusarium pathogens and biological modification of trichothecenes in cereal grains. Toxins 9:408. https://doi.org/10.3390/toxins9120408

Yen AL (2009) Edible insects: traditional knowledge or western phobia? Entomol Res 39:289-298. https://doi.org/10.1111/j.1748-5967.2009.00239.x

Yen AL (2015) Insects as food and feed in the Asia pacific region: current perspectives and future directions. J Insects as Food and Feed 1:33-55. https://doi.org/10.3920/jiff2014.0017

Yi L, Lakemond CM, Sagis LM, Eisner-Schadler V, van Huis A, van Boekel MA (2013) Extraction and characterisation of protein fractions from five insect species. Food Chem 141:3341-3348. https://doi.org/10.1016/j.foodchem.2013.05.115

Zielińska E, Baraniak B, Karaś M, Rybczyńska K, Jakubczyk A (2015) Selected species of edible insects as a source of nutrient composition. Food Res Int 77:460-466. https://doi.org/10.1016/j.foodres.2015.09.008

List of figures

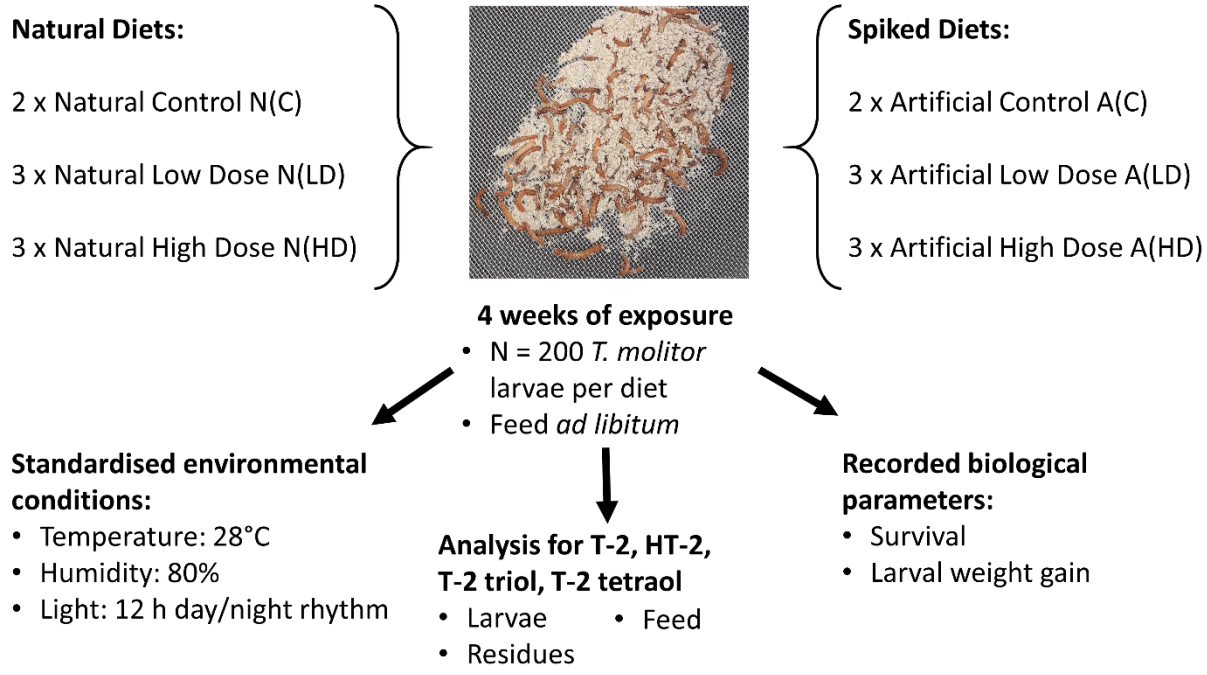

Figure 1 Feeding experiment design: Each diet contained n = 200 *T. molitor* larvae fed on 6 g designated feed for 4 weeks. For diet preparation and concentration see. Table 1.

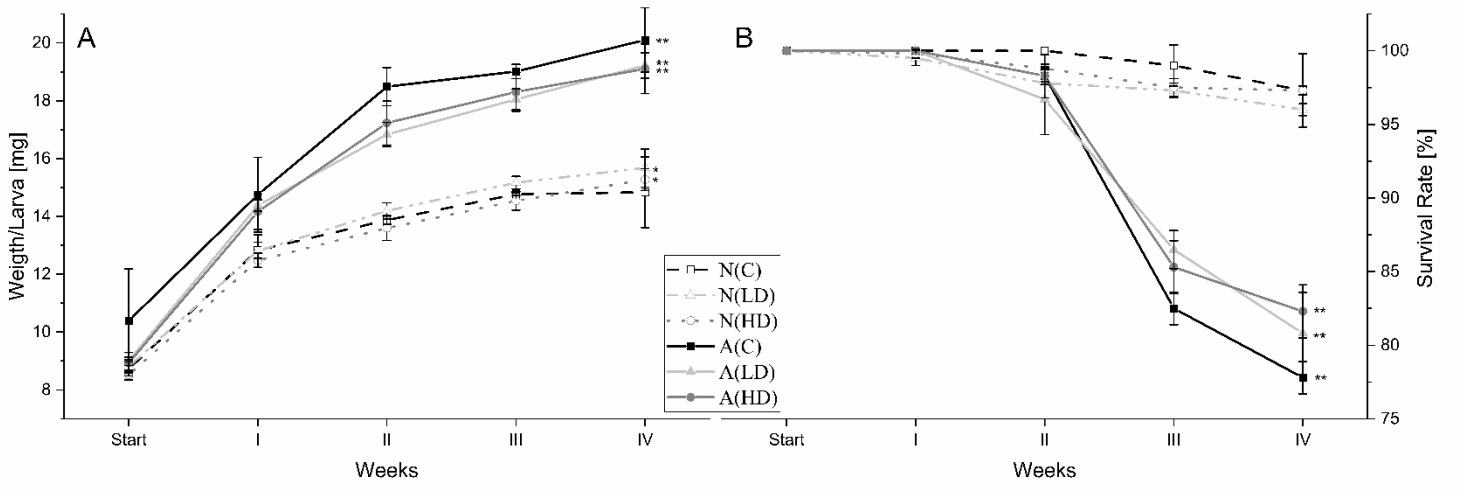

Figure 2 Average larval growth (A) and survival rates (B) for the different diet groups during 4 weeks of exposure to trichothecene contaminated oat flakes (*= P <0.05, **=P <0.001; linear regression model).

Tables

Table 1 Different diets, amounts of T-2 and HT-2 toxin, and nutrient composition

Diet	Code	Biological replicates	T-2 [µg/kg]	HT-2 [µg/kg]	Dry Matter [%]	Protein [%] [d]	Total Energy [MJ/kg]
Natural Control[a]	N(C)	2	0	0	90.1	14.3	17.8
Natural Low Dose [b]	N(LD)	3	88.8 ± 11.6	11.1± 3.0	90.6	15.5	18.1
Natural High Dose [b]	N(HD)	3	262.3 ± 47.3	26.0 ± 6.6	91.1	14.3	17.9
Artificial Control[a]	A(C)	2	0	0	93.3	14.5	18.3
Artificial Low Dose[c]	A(LD)	3	53.9 ± 3.8	51.6 ± 0.9	95.7	15.8	18.7
Artificial High Dose[c]	A(HD)	3	139.8 ± 7.6	120.9 ± 2.2	95.9	14.4	18.7

[a] Uncontaminated ground oat flakes for human consumption
[b] Naturally moulded oat flakes (*F. sporotrichioides* var. *minus* Wollenweber 1930); contents adjusted by mixing and homogenization with uncontaminated ground oat flakes
[c] Ground oat flakes artificially contaminated with standards of T-2/HT-2 toxin
[d] Protein content on a dry matter basis

Table 2 Performance parameters of the LC-MS/MS-method including recovery rates (n= 6 replicates) and precision

Toxin	Matrix	LOD [µg/kg]	LOQ [µg/kg]	Level of fortification [µg/kg]	Recovery [%]	RSD_r [%]	RSD_R [%]
T-2	Larvae	4.6	15.3	75	107.6	0.1	6.9
				187.5	112	1.3	7.1
	Oat flakes	3.3	11	75	101.2	4	18.5
				187.5	101.2	4	8.4
	Residue	2.1	7	75	115.4	6.1	8.6
				187.5	102.7	8.3	1.9
HT-2	Larvae	8.4	27.9	75	101.8	0.1	11
				187.5	134.3	7.2	10.2
	Oat flakes	12	39.7	75	86.1	7.7	17.3
				187.5	79.8	6.4	10.3
	Residue	20.5	68.2	75	97.7	7.5	16.9
				187.5	100	6.5	8.9
T-2 triol	Larvae	29.6	98.8	75	104.3	3.4	9.6
				187.5	105.9	4.9	7
	Oat flakes	9.5	31.5	75	75.9	11.4	25.4
				187.5	72.8	10	27.1
	Residue	69	184.4	187.5	116.1	16.3	9.5
				300	95.8	3.7	11.5
T-2 tetraol	Larvae	24.6	82.4	75	50.1	15.9	8.5
				187.5	48.5	9.5	11
	Oat flakes	30	102	75	60.1	24.1	11.1
				187.5	59.4	7.4	14.1
	Residue	25	83.7	75	50.1	22.8	10.6
				187.5	50	7.3	5.8

LOD limit of detection*; LOQ* limit of quantification;
RSDr Intraday precision was determined as relative standard deviation by 6 replicates for each sample material and concentration*;*
RSDR Interday precision was determined as relative standard deviation with one replication on 5 consecutive days.

Table 3 Larval weight gain and survival during the 4-week feeding experiment

Diet	Code/Replicate No.	Weight gain [%]	Mean ± SD [%]	Weight gain RSD [%]	Survival [%]	Mean ± SD [%]
Natural Control (n=2)	N(C) 1	61.4	69.8 ± 11.8	16.8	94.5	97.3 ± 3.9
	N(C) 2	78.1			100	
Natural Low Dose (n=3)	N(LD) 1	87.4			94.5	
	N(LD) 2	75.2	78.1 ± 8.2	10.4	97.5	96 ± 1.5
	N(LD) 3	71.9			96	
Natural High Dose (n=3)	N(HD) 1	80.7			98.5	
	N(HD) 2	77.4	79.3 ± 1.7	2.2	98	97.3 ± 1.6
	N(HD) 3	79.9			95.5	
Artificial Control (n=2)	A(C) 1	79	95.5 ± 23.2	24.3	76.5	77.8 ± 1.8
	A(C) 2	111.9			79	
Artificial Low Dose (n=3)	A(LD) 1	116.9			87	
	A(LD) 2	116.4	113.3 ± 5.8	5.1	77.5	80.8 ± 5.3
	A(LD) 3	106.7			78	
Artificial High Dose (n=3)	A(HD) 1	113.3			82.5	
	A(HD) 2	112.9	113.8 ± 1.3	1.1	84	82.3 ± 1.8
	A(HD) 3	115.2			80.5	

Table 4 Amounts of type A trichothecenes in sample material of the different diets

Sample material	Diet	Code	T-2 [µg/kg]	HT-2 [µg/kg]	T-2 triol [µg/kg]	T-2 tetraol [µg/kg]
Larvae	Natural Control	N(C)	nd	nd	nd	nd
	Natural Low Dose	N(LD)	nd	nd	nd	nd
	Natural High Dose	N(HD)	nd	nd	nd	nd
	Artificial Control	A(C)	nd	nd	nd	nd
	Artificial Low Dose	A(LD)	nd	nd	nd	nd
	Artificial High Dose	A(HD)	nd	nd	nd	nd
Dead larvae[a]	Natural Control	N(C)	nd	nd	nd	nd
	Natural Low Dose	N(LD)	7.7[c]	nd	nd	nd
	Natural High Dose	N(HD)	44.2	nd	nd	nd
	Artificial Control	A(C)	nd	nd	nd	nd
	Artificial Low Dose	A(LD)	7.7[c]	nd	nd	nd
	Artificial High Dose	A(HD)	7.7[c]	nd	nd	nd
Residue[b]	Natural Control	N(C)	nd	nd	nd	nd
	Natural Low Dose	N(LD)	58.6[d]	nd	nd	nd
	Natural High Dose	N(HD)	135.8[d]	nd	nd	nd
	Artificial Control	A(C)	nd	nd	nd	nd
	Artificial Low Dose	A(LD)	29.7	34.1[c]	nd	nd
	Artificial High Dose	A(HD)	51.1	34.1[c]	nd	nd

nd not detected (< LOD)
[a] *Dead larvae* larvae, that died during 4-week exposure
[b] *residue* mixture of oat flour and larval faeces
c Levels < LOQ were considered as 0.5 LOQ
d Sample pooled to achieve sufficient material for measurement

Electronic supplementary material

Online Resource 1 Retention times and mass spectrometric conditions for determination of investigated trichothecenes.

Article title: Distribution of T-2 toxin and HT-2 toxin during experimental feeding of yellow mealworm (Tenebrio molitor)

Journal name: Mycotoxin Research

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Compound	Abbrev.	Retention time[1] [min]	Precursor ion [m/z]	Quantifier ion [m/z]	Qualifier ion [m/z]	DP [V]	CE [eV] (quant/qual)	CXP [V] (quant/qual)	Ion ratio[2] (qual / quant)
T-2 Toxin	T-2	4.9	484.3	305.2	245.1	61	21/19	18/14	0.79
HT-2 Toxin	HT-2	4.5	442.2	263.1	215.1	51	19/21	14. Dez	0.94
T-2 Triol	Triol	4.3	400.3	215.2	281.4	41	17/13	Okt 26	0.31
T-2 Tetraol	Tetraol	1.4	316.2	215.1	233.2	46	15. Sep	20/44	0.37

Entrance potential (EP) = 10 V for all analytes. Abbreviations: DP: declustering potential, CE: collision energy, CXP: cell exit potential

[1] Determined using the final HPLC conditions; [2] Mean of three injections of a standard solution (c = 50 ng/mL)

4 Discussion

As explained in detail in section “Discussion” of the Publication (see section 3.), *T. molitor* larvae have been affected both by the trichothecenes as well as by the diet preparation itself. Larval growth was positively influenced by the addition of trichothecene containing *F. sporotrichioides*, but the toxin amounts seemed to have no effect on larval survival within the two diet groups. The detection of T-2 in dead, but not in living larvae indicated a yet unknown metabolic or biotransformation process in the larvae.

The T-2 concentrations, detected in dead larvae, as well as T-2 and HT-2 concentrations in residue are -compared to toxin levels regulated in 2013/165/EU (EC 2013) - for cereals quite low. But to evaluate the remaining cytotoxicity of the sample material and to safely exclude any potential risk to humans or animals, further investigations using effect based bioassays such as the methylthiazoltetrazolium (MTT) -cell culture assay (Gareis 2006; Hanelt et al. 1994) could deliver additional information on the safety and would be another technique to compare the different diets. This bioassay enables the quick and sensitive toxicity determination of extracted sample material on mammalian cells (Swine Kidney cell line). Due to its low specificity, the remaining toxicity even of yet unknown metabolites on mammalian cells may be detected.

Especially such unknown metabolites are from scientific interest regarding the biotransformation of insects. As mentioned above, the animals of the class of insects have a different anatomy and physiology compared to mammals. Therefore, a mammal-like degradation of trichothecenes divided in phase-I-/-II -metabolism appears unlikely, not least because of the lack of the anatomical structures required for these processes, such as liver cells including the necessary enzymes.

Moreover, insects are able to detoxify noxious agents in a way that is impossible for mammals. Bass et al. (2015) reviewed the biochemical and molecular mechanisms of insects involved in the resistance against neonicotinoid insecticides and revealed insect unique processes of detoxification. Additionally, Ivie et al. (1983) investigated the larvae of a certain insect family, the *Papilionidae*. These larvae feed successfully and preferentially on plants that contain psoralens, without any signs of intoxication. Psoralens (linear furocoumarins) are photoactive compounds that readily alkylate DNA when activated by longwave ultraviolet light and pose significant toxicological risks to man and other organisms. This second study reveals another

important aspect related to the mycotoxin biotransformation in insects: The specific family or species adaption to their habitat and biological surrounding.
As different metabolism of trichothecenes is described for different mammal orders (Doi et al., 2006), it is more than likely that in a such diverse animal group like insects, different biotransformation strategies for detoxification of noxious agents have evolved.

As mentioned above *T. molitor* larvae have been affected by the trichothecenes as well as by the diet preparation itself in the presented study, these results pointing to new working hypothesis and study design adaptions for further upcoming studies. First adaptions should be made regarding the form, the diets were provided to the larvae.
In the current feeding experiment the feed conversion of the different diets could not be recorded, because the larvae additionally crushed the initially, already very fine, oat flour during the 4 weeks feeding trail and afterwards a separation from their residues was no longer possible. Therefore, the flour should be pelletized after contamination, this would ensure that the larvae are still able to eat the prepared diets, but diet fragmentation is small enough for an adequate separation from the residue.

Additionally, this procedure would enable separate LC-MS/MS analysis of remaining oat flour and residue for the investigated trichothecenes. A separate determination of trichothecene levels is from scientific interest as e.g., Niermans et al. (2019) described differing zearalenone concentrations in residue and remaining diet material, indicating metabolic transformations of mycotoxins in the larvae.

As the oats of the spiked diet were prepared as a slurry in distilled water and heated, it is possible that vitamins or other sensitive micronutrients were degraded or at least their content diminished. Therefore, it seems appropriate to omit the heat step and proceed directly with lyophilisation, in order to reduce the potential influencing factors to a minimum. The applied dilution of the reference standard solutions in water and the mixing with the oats appears unavoidable to ensure a homogeneous distribution of trichothecenes in the prepared contaminated diets.

As entomophagy in Europe is struggling with consumer refusal and safety concerns (Hamerman, 2016; Tan et al., 2016), the result of the presented study could be used to mitigate these food safety concerns. As *T. molitor* is one of the edible insect species that already has a comparable high European consumer acceptance (Bednářová et al., 2013;

Hartmann et al., 2015), these findings could contribute to change the consumers and producers current focus away from concerns to the beneficial aspects of entomophagy. Especially since 15.3 % of women and 15.6 % of men in Europe are suffering from obesity (Lange and Finger, 2017), the inclusion of insects into the diet of Europeans could have positive effects both for each individual and for the burdened health systems of the member countries. The relatively high protein content- although discussed controversially (Jonas-Levi and Martinez, 2017) - with low fat content is of particular importance here.

In addition to the nutritional benefits, economical, and ecological aspects of edible insects farming also have to be considered. Compared to other livestock, edible insects are less resource demanding by an even higher food conversion (Dobermann et al., 2017; Miglietta et al., 2015; van Huis et al., 2015). A very high selling price of e.g., 49.50€ per 100g Grasshopper powder (kreca®, June 2020), is also of economic interest. The present study, in line with several other mycotoxin related feeding studies (Davis and Schieffer, 1982; Niermans et al., 2019; Ochoa Sanabria et al., 2019; Van Broekhoven et al., 2017), pointing towards *T. molitor* larvae (harvested as sane, living organisms) being a potential degrader of T-2/HT-2 as well as a safe utilizer of trichothecene contaminated grains. Indicating the possibility that spoiled grains could be used as diet for food-producing insects, these findings represent a further - economically and ecological very important - aspect for the insect industry.
This could lead to a decreased waste of resources by a simultaneously sustainable food or feed production. Insects as feed appear in this context as almost perfect solution, as they could be rear on spoiled grains, which are found on nearly every farm, and afterwards used as concentrated feed for livestock e.g., pigs or poultry. Although such a procedure is currently prohibited in the EU, it offers an outlook, especially regarding the United Nations Sustainable Development Goals (no poverty, zero hunger, good health and well-being) (FAO 2009).

In conclusion, the presented study demonstrated that *T. molitor* larvae have been affected concerning weight and survival by *F. sporotrichioides* and type-A trichothecenes as well as the diet preparation itself.
In a wider perspective these findings, being in accordance with several other mycotoxin related feeding experiments on *T. molitor* (Niermans et al., 2019; Van Broekhoven et al., 2017), reaffirm the expectations of edible insects to potentially contribute to meet the challenges of the future. As biotransformation of mycotoxins has to be considered to be different compared to mammals, these unknown pathways have to be further investigated

and the toxicity of insect metabolites should be evaluated with appropriate methods, like a MTT-Bioassay (Gareis, 2006). Nevertheless, the presented results indicated *T. molitor* larvae (harvested as fit, living organisms) being a potential degrader of T-2/HT-2 as well as a safe utilizer of contaminated grains, especially when containing trichothecenes.

5 Zusammenfassung

Insekten sind mit über einer Million beschriebenen und mit bis zu 2,5 Millionen geschätzten Arten (Storch 2018; Zhang 2011) die artenreichste Gruppe im Tierreich. Sie kommen auf allen Kontinenten und Ozeanen der Welt vor und besiedeln verschiedenste Habitate. Über 2100 Insektenarten gelten momentan als zum menschlichen Verzehr geeignet. Aufgrund ihres hohen Proteinanteils und der effizienten Futterumsetzung gelten Insekten als eine potenzielle Nahrungsmittelquelle, um die ersten drei Ziele für nachhaltige Entwicklung (keine Armut, kein Hunger, gute Gesundheit und Wohlbefinden) der Vereinten Nationen zu erreichen (FAO, 2009). Innerhalb der Europäischen Union müssen essbare Insekten gemäß der Verordnung (EU) 2015/2283 als neuartige Lebensmittel („Novel Food“) zugelassen werden und unterliegen demzufolge den Anforderungen an Hygiene und Sicherheit von Lebensmitteln, dazu gehört unter anderem auch die Einhaltung behördlicher Grenzwerte für Mykotoxine. Da Substrate, die bei der Fütterung von Insekten eingesetzt werden, häufig anfällig für einen Befall mit Trichothecene produzierenden Schimmelpilzen der Gattung *Fusarium* sind, besteht Grund zu der Annahme, dass es zu einer Mykotoxinkontamination sowohl der Futtermittel als auch in weiterer Folge der Insekten selbst kommen könnte.

Hinsichtlich der Übertragung von Mykotoxinen aus verschimmelten Substraten auf Insekten wurden mehrere Fütterungsexperimente mit Larven des gelben Mehlwurms (*Tenebrio molitor*) durchgeführt, die auf eine Biotransformation von Trichothecenen, z.B. Zearalenon (Niermans et al., 2019) oder Deoxynivalenol (Ochoa Sanabria et al., 2019) durch die Larven hindeuten. Umfassende Untersuchungen zum Metabolismus und zum Verbleib von Typ A Trichothecenen in Insekten, insbesondere in *T. molitor*, liegen jedoch bisher nicht vor.

Das Ziel dieser Studie war es daher, den Einfluss von T-2- und HT-2-Toxin auf den Lebenszyklus von *T. molitor* Larven zu untersuchen und das Vorkommen von T-2, HT-2, T-2 triol und T-2 tetraol in den Larven nachzuweisen. In einer vierwöchigen Fütterungsstudie wurden *T molitor*-Larven entweder auf natürlichem (mit *Fusarium sporotrichioides* befallenem) oder künstlich mit diesen Trichothecenen kontaminiertem Hafer gehalten. Diese Haferdiäten wurden jeweils in den Konzentrationsstufen 100 und 250 µg/kg (Summe aus T-2 und HT-2) hergestellt. Im Verlauf des Experiments wurden die Sterblichkeit und die Gewichtszunahme der Larven erfasst. Nach Ablauf der vier Wochen wurde das Probenmaterial mittels LC-MS/MS

Analytik auf das Vorkommen der vier Trichothecene T-2, HT-2, T-2 triol und T-2 tetraol hin untersucht.

Während des Experiments unterschieden sich die verschiedenen Diäten in Hinblick auf die Larvenentwicklung: Larven, die mit künstlich kontaminiertem Hafer gefüttert wurden (einschließlich der negativ Kontrollen), zeigten hierbei eine um bis zu 44 % höhere Gewichtszunahme als Larven auf dem natürlich kontaminierten Futter. Allerdings wurde bei ersteren Larven auch eine 16 % höhere Sterblichkeit beobachtet. Nach Abschluss des Experiments wurden in den überlebenden Mehlwurmlarven keine nachweisbaren Gehalte der untersuchten Trichothecene mehr festgestellt. In den Larven, die während der Studie starben, als auch in den Rückständen (nicht trennbare Mischung aus Kot und Hafer) der verschiedenen Diäten wurde jedoch T-2 und HT-2 nachgewiesen. Dies war bei beiden Populationen der unterschiedlichen Futtermittelkontaminationsquellen der Fall. Diese Ergebnisse zeigen, dass *T. molitor* Larven von Typ A Trichothecenen beeinflusst werden. In Hinblick auf die Lebensmittelsicherheit bleibt festzuhalten, dass die lebenden Larven dennoch als sichere Verwerter von T-2/HT-2-kontaminiertem Hafer angesehen werden können.

6 Summary

Insects are the most diverse animal group on earth, with over one million described and up to 2.5 million estimated species (Stork, 2018; Zhang, 2011). They are abundant on all continents and oceans of the world and appear in most diverse habitats. Over 2000 species are referred as edible. Due to high-quality protein and high biomass conversion, edible insects are regarded as a potential contributor to the first three Sustainable Development Goals of the United Nations (no poverty, zero hunger, good health and well-being) (FAO, 2009). Within the EU, edible insects need to be approved as Novel Food according to Regulation (EU) 2015/2283 and have to fulfil food safety requirements like regulatory limits for mycotoxins. Substrates used for feeding insects are receptive for growth of *Fusarium* spp. and, consequently, a contamination with mycotoxins, especially trichothecenes.

Regarding the transfer of mycotoxins from mouldy substrates to insects several trichothecene feeding trails have been conducted with larvae of the Yellow Mealworm (*Tenebrio molitor*), pointing towards degradation of trichothecenes e.g., zearalenone (Niermans et al., 2019) or deoxynivalenol (Ochoa Sanabria et al., 2019) by the larvae. However, comprehensive investigations on the metabolism and fate of type A trichothecenes in insects, especially in *T. molitor,* are not available so far.

Therefore, the current study aimed to investigate the influence of T-2 and HT-2 toxin on *T. molitor* larvae's life cycle and to determine an occurrence of T-2, HT-2, T-2 triol and T-2 tetraol in the larvae. In a 4-week feeding study, *T. molitor* larvae were kept either on naturally (oats moulded with *Fusarium sporotrichioides*) or artificially contaminated oats (each two levels with approximately 100 and 250 µg/kg, sum T-2/HT-2). Weight gain and survival were monitored, and mycotoxins were determined using LC-MS/MS.

Larval development varied between the diets, resulting in 44% more weight gain in the artificially contaminated diets. On the contrary, these groups also had a lower survival rate by 16 %. Measurements after harvest revealed no detectable levels of the investigated trichothecenes in the surviving mealworm larvae. However, T-2 and HT-2 were found in the residues and larvae that died during the study, both in naturally and artificially contaminated diets. Therefore, the results revealed an impact of type A trichothecenes on T. molitor, still indicating that the larvae may be safe utilizers of T-2/HT-2-contaminated oats.

7 References

APRD, Arthropod Pesticide Resistance Database. (2020) Imidacloprid Case Reports 2000-2020. https://www.pesticideresistance.org/search.php. Accessed: 30.08.2020

BARROSO, F. G., DE HARO, C., SÁNCHEZ-MUROS, M.-J., VENEGAS, E., MARTÍNEZ-SÁNCHEZ, A. & PÉREZ-BAÑÓN, C. (2014) The potential of various insect species for use as food for fish. Aquaculture 422-423, 193-201

BASS, C., DENHOLM, I., WILLIAMSON, M. S. & NAUEN, R. (2015) The global status of insect resistance to neonicotinoid insecticides. Pesticide Biochemistry and Physiology 121, 78-87

BAUER, J., GAREIS, M. & GEDEK, B. (1989) Metabolism of the trichothecenes T-2 toxin, diacetoxyscirpenol, and deoxynivalenol by farm animals. In: Chełkowski J (ed) Topics in Secondary Metabolism, vol 2, Fusarium: Mycotoxins, Taxonomy, Pathogenicity. Elsevier, New York, pp 139-165.

BEER, M. (2017) Produktion und Verarbeitung von Insekten zur Verwendung als Lebensmittel. https://www.blv.admin.ch/dam/blv/de/dokumente/lebensmittel-und-ernaehrung/rechts-und-vollzugsgrundlagen/hilfsmittel-vollzugsgrundlagen/informationsschreiben-neu/lme-informationsschreiben-2017-1.pdf.download.pdf/Informationsschreiben_2017_1__Produktion_und_Verarbeitung_von_Insekten_zur_Verwendung_als_Lebensmittel.pdf. Accessed: 07.05.2020

BERTHILLER, F., BRERA, C., IHA, M., KRSKA, R., LATTANZIO, V., MACDONALD, S., MALONE, R., MARAGOS, C., SOLFRIZZO, M. & STRANSKA-ZACHARIASOVA, M. (2017) Developments in mycotoxin analysis: an update for 2015-2016. World Mycotoxin Journal 10, 5-29

BFR, Bundesamt für Risikoanalysen (2020) TDI / TWI / TMI – daily, weekly or monthly: the tolerable intake of toxins is clearly defined. https://www.bfr.bund.de/en/a-z_index/tdi-187181.html. Accessed: 12.06.2020

BONDY, G. S. & PESTKA, J. J. (2000) Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health Part B: Critical Reviews 3, 109-143

BOSCH, G., FELS-KLERX, H. J. V., RIJK, T. C. & OONINCX, D. (2017) Aflatoxin B1 tolerance and accumulation in black soldier fly larvae (Hermetia illucens) and yellow mealworms (Tenebrio molitor). Toxins (Basel) 9, 185

BOTTALICO, A. & PERRONE, G. (2002) Toxigenic Fusarium species and mycotoxins associated with head blight in small-grain cereals in Europe. In Mycotoxins in plant disease, Berlin, Springer. 611-624

CAMENZULI, L., VAN DAM, R., DE RIJK, T., ANDRIESSEN, R., VAN SCHELT, J. & VAN DER FELS-KLERX, H. J. I. (2018) Tolerance and excretion of the mycotoxins aflatoxin B(1), zearalenone, deoxynivalenol, and ochratoxin A by Alphitobius diaperinus and Hermetia illucens from contaminated substrates. Toxins 10, 91

CAVRET, S. & LECOEUR, S. (2006) Fusariotoxin transfer in animal. Food and Chemical Toxicology 44, 444-453

COFFIN, J. & COMBS JR, G. (1981) Impaired vitamin E status of chicks fed T-2 toxin. Poultry science 60, 385-392

COLE, R. J., SCHWEIKERT, M. A. & JARVIS, B. B. (2003) Handbook of secondary fungal metabolites, 3-volume set. New York, Elsevier

CONRADY-LORCK, S., GAREIS, M., FENG, X. C., AMSELGRUBER, W., FORTH, W. & FICHTL, B. (1988) Metabolism of T-2 toxin in vascularly autoperfused jejunal loops of rats. Toxicology and applied pharmacology 94, 23-33

CREWS, C. (2015) Analysis of ergot alkaloids. Toxins 7, 2024-2050

CREWS, C., BERTHILLER, F. & KRSKA, R. (2010) Update on analytical methods for toxic pyrrolizidine alkaloids. Analytical and bioanalytical chemistry 396, 327-338

DAVIS, G. R. F. & SCHIEFFER, H. B. (1982) Effects of dietary T2 toxin concentrations fed to larvae of the yellow mealworm at three dietary protein levels. Comparative Biochemistry and Physiology 73, 13-16

DEFOLIART, G. R. (1995) Edible insects as minilivestock. Biodiversity & Conservation 4, 306-321

DOBERMANN, D., SWIFT, J. & FIELD, L. (2017) Opportunities and hurdles of edible insects for food and feed. Nutrition Bulletin 42, 293-308

DOHNAL, V., JEZKOVA, A., JUN, D. & KUCA, K. (2008) Metabolic pathways of T-2 toxin. Current drug metabolism 9, 77-82

DOI, K., SHINOZUKA, J. & SEHATA, S. (2006) T-2 toxin and apoptosis. Toxicologic Pathology 19, 15-27

EDI, Eidgenössisches Departement des Inneren (2016) Verordnung des EDI über die Hygiene beim Umgang mit Lebensmitteln. https://www.admin.ch/opc/de/classified-compilation/20143394/index.html. Accessed: 22.09.2020

EC, European Commission (2005) Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Offical Journal of the European Commission L 338/1, Last consolidated version available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2073

EFSA, European Food Safety Authority (2011) Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA Journal 9, 2481

EFSA, European Food Safety Authority (2014) Scientific Opinion on the risks for human and animal health related to the presence of modified forms of certain mycotoxins in food and feed. EFSA Journal 12, 3916

ELHASSAN, M., WENDIN, K., OLSSON, V. & LANGTON, M. (2019) Quality aspects of insects as food-nutritional, sensory, and related concepts. Foods 8, 3

EU, European Union (2006) Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Offical Journal of the European Union 70, 12-34, Last consolidated version available from: https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32006R0401

EU, European Union (2011) Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive Text with EEA relevance. Offical Journal of the European Union L 54, 1-251, Last consolidated version available from: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex:32011R0142

EU, European Union (2013) Commission Recommendation of 27 March 2013 on the presence of T-2 and HT-2 toxin in cereals and cereal products. Offical Journal of the European Union L 91, 12-15, Last consolidated version available from: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:091:0012:0015:EN:PDF

EU, European Union (2017) Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. Offical Journal of the European Union L 138, 92-116, Last consolidated version available from: https://eur-lex.europa.eu/legal-content/DE/TXT/?uri=CELEX%3A32017R0893

FAO, Food and Agriculture Organization of the United Nations (2009) How to feed the world in 2050. https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf. Accessed 25 August 2020

FAO, IFAD, UNICEF, W. & WHO (2019) The state of food security and nutrition in the world 2019. http://www.fao.org/3/ca5162en/ca5162en.pdf . Accessed: 22.09.2020

FINKE, M. D. (2002) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biology 21, 269-285

FUEREDER, L., SCHUETZ, C., WALLINGER, M. & RAINER, B. (2001) Physico-chemistry and aquatic insects of a glacier-fed and a spring-fed alpine stream. Freshwater Biology 46, 1673-1690

GAREIS, M. (2006) Diagnostischer Zellkulturtest (MTT-test) für den Nachweis von zytotoxischen Kontaminanten und Rückständen. Journal für Verbraucherschutz und Lebensmittelsicherheit 1, 354-363

GDANETZ, K. & TRAIL, F. (2017) The wheat microbiome under four management strategies, and potential for endophytes in disease protection. Phytobiomes 1, 158-168

GERBER, G. (1975) Reproductive behaviour and physiology of Tenebrio molitor (Coleoptera: Tenebrionidae): II. Egg development and oviposition in young females and the effects of mating. The Canadian Entomologist 107, 551-559

GERBER, G. & SABOURIN, D. (1984) Oviposition site selection in Tenebrio molitor (Coleoptera: Tenebrionidae). The Canadian Entomologist 116, 27-39

GOTTSCHALK, C., BARTHEL, J., ENGELHARDT, G., BAUER, J. & MEYER, K. (2009) Simultaneous determination of type A, B and D trichothecenes and their occurrence in cereals and cereal products. Food Additives & Contaminants: Part A 26, 1273-1289

HAMERMAN, E. J. (2016) Cooking and disgust sensitivity influence preference for attending insect-based food events. Appetite 96, 319-326

HANBOONSONG, Y. & JAMJANYA, T. D., PATRICK B. (2013) Six-legged livestockedible insect farming, collection and marketing in Thailand. http://www.fao.org/3/i3246e/i3246e00.htm. Accessed: 22.09.2020

HARTMANN, C., SHI, J., GIUSTO, A. & SIEGRIST, M. (2015) The psychology of eating insects: A cross-cultural comparison between Germany and China. Food Quality and Preference 44, 148-156

IKAWA, T., HIDEHIKO, O., HOSHIZAKI, S., KAMIKADE, T. & CHENG, L. (2004) Distribution of the oceanic insects Halobates (Hemiptera: Gerridae) off the south coast of Japan. Entomological Science 7, 351-357

IMATHIU, S. (2019) Benefits and food safety concerns associated with consumption of edible insects. NFS Journal

IPIFF, International Platform of Insects for Food and Feed (2019) IPIFF Guidance the provision of food information to consumers Edible insect-based products. https://ipiff.org/wp-content/uploads/2019/12/IPIFF-Guide-on-Good-Hygiene-Practices.pdf. Accessed 19 February 2020

IVIE, G. W., BULL, D. L., BEIER, R. C., PRYOR, N. W. & OERTLI, E. H. (1983) Metabolic detoxification: mechanism of insect resistance to plant psoralens. Science 221, 374-376

JAKOBY, W. B. & ZIEGLER, D. M. (1978) The glutathione S-transferases: a group of multifunctional detoxification proteins. Adv Enzymol Relat Areas Mol Biol 46, 383-414

JECFA, Joint FAO/WHO Expert Committee on Food Additives (2001) Safety evaluation of certain mycotoxins in food, http://www.fao.org/3/a-bc528e.pdf. Accessed: 22.09.2020

JOFFE, A. Z. (1986) Fusarium species: their biology and toxicology. New York, John Wiley & Sons

JONAS-LEVI, A. & MARTINEZ, J. J. I. (2017) The high level of protein content reported in insects for food and feed is overestimated. Journal of Food Composition and Analysis 62, 184-188

JONGEMA, Y. (2017) Worldwide list of recorded edible insects. https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm, Wageningen University & Research. Accessed: 19.2.2020

KARUNKER, I., BENTING, J., LUEKE, B., PONGE, T., NAUEN, R., RODITAKIS, E., VONTAS, J., GORMAN, K., DENHOLM, I. & MORIN, S. (2008) Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of Bemisia tabaci (Hemiptera: Aleyrodidae). Insect biochemistry and molecular biology 38, 634-644

KIEROŃCZYK, B., SYPNIEWSKI, J., RAWSKI, M., CZEKAŁA, W., ŚWIĄTKIEWICZ, S. & JÓZEFIAK, D. (2019) From waste to sustainable feed material: the effect of Hermetia illucens oil on the growth performance, nutrient digestibility, and gastrointestinal tract morphometry of broiler chickens. Annals of Animal Science 20, 157-177

KLUNDER, H. C., WOLKERS-ROOIJACKERS, J., KORPELA, J. M. & NOUT, M. J. R. (2012) Microbiological aspects of processing and storage of edible insects. Food Control 26, 628-631

KOHANSKI, M. A., THARAKAN, A., LONDON, N. R., LANE, A. P. & RAMANATHAN JR, M. (2017) Bactericidal antibiotics promote oxidative damage and programmed cell death in sinonasal epithelial cells. In International forum of allergy & rhinology, Wiley Online Library. 359-364

KRECA® (2020) https://www.krecafood.com/insect-powder/grasshoppers/. Accessed: 06.06.2020

KRSKA, R., BAUMGARTNER, S. & JOSEPHS, R. (2001) The state-of-the-art in the analysis of type-A and-B trichothecene mycotoxins in cereals. Fresenius' journal of analytical chemistry 371, 285-299

KUMAGAI, S. & SHIMIZU, T. (1988) Effects of Fusarenon-X and T-2 toxin on intestinal absorption of monosaccharide in rats. Archives of toxicology 61, 489-495

LANGE, C. & FINGER, J. D. (2017) Gesundheitsverhalten in Europa–Vergleich ausgewählter Indikatoren für Deutschland und die Europäische Union. Journal of Health Monitoring 2

LI, G., XIA, X., ZHAO, S., SHI, M., LIU, F. & ZHU, Y. (2020) The physiological and toxicological effects of antibiotics on an interspecies insect model. Chemosphere 248, 126019

LI, Y., LUO, X., YANG, S., CAO, X., WANG, Z., SHI, W. & ZHANG, S. (2014) High specific monoclonal antibody production and development of an ELISA method for monitoring T-2 toxin in rice. Journal of Agricultural and Food Chemistry 62, 1492-1497

LI, Y., WANG, Z., BEIER, R. C., SHEN, J., DE SMET, D., DE SAEGER, S. & ZHANG, S. (2011) T-2 toxin, a trichothecene mycotoxin: review of toxicity, metabolism, and analytical methods. Journal of Agricultural Food Chemistry 59, 3441-3453

LI, Z., YANG, D., HUANG, M., HU, X., SHEN, J., ZHAO, Z. & CHEN, J. (2012) Chrysomya megacephala (Fabricius) larvae: a new biodiesel resource. Applied Energy 94, 349-354

LUDWIG, D. & FIORE, C. (1960) Further studies on the relationship between parental age and the life cycle of the mealworm, Tenebrio molitor. Annals of the Entomological Society of America 53, 595-600

LUNDY, M. E. & PARRELLA, M. P. (2015) Crickets are not a free lunch: protein capture from scalable organic side-streams via high-density populations of Acheta domesticus. PLOS One 10

MANZANO-AGUGLIARO, F., SANCHEZ-MUROS, M. J., BARROSO, F. G., MARTÍNEZ-SÁNCHEZ, A., ROJO, S. & PÉREZ-BAÑÓN, C. (2012) Insects for biodiesel production. Renewable and Sustainable Energy Reviews 16, 3744-3753

MCCORMICK, S. P., STANLEY, A. M., STOVER, N. A. & ALEXANDER, N. J. (2011) Trichothecenes: from simple to complex mycotoxins. Toxins 3, 802-814

MEZES, M., BARTA, M. & NAGY, G. (1999) Comparative investigation on the effect of T-2 mycotoxin on lipid peroxidation and antioxidant status in different poultry species. Research in veterinary science 66, 19-23

MIGLIETTA, P. P., DE LEO, F., RUBERTI, M. & MASSARI, S. (2015) Mealworms for food: a water footprint perspective. Water 7, 6190-6203

MIROCHA, C. & PATHRE, S. (1973) Identification of the toxic principle in a sample of poaefusarin. Applied and Environmental Microbiology 26, 719-724

MONTOWSKA, M., KOWALCZEWSKI, P. L., RYBICKA, I. & FORNAL, E. (2019) Nutritional value, protein and peptide composition of edible cricket powders. Food Chemistry 289, 130-138

MUNSCH, N. & MÜLLER, W. E. (1980) Effects of T2 toxin on DNA polymerases and terminal deoxynucleotidyl transferase of Molt4 and Nu8 cell lines. Immunopharmacology 2, 313-318

NAKAGAKI, B. J. & DEFOLIART, G. R. (1991) Comparison of diets for mass-rearing Acheta domesticus (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. Journal of Economic Entomology 84, 891-896

NAUEN, R., STUMPF, N. & ELBERT, A. (2002) Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type Bemisia tabaci (Hemiptera: Aleyrodidae). Pest Management Science 58, 868-875

NIERMANS, K., WOYZICHOVSKI, J., KRONCKE, N., BENNING, R. & MAUL, R. (2019) Feeding study for the mycotoxin zearalenone in yellow mealworm (Tenebrio molitor) larvae-investigation of biological impact and metabolic conversion. Mycotoxin Research 35, 231–242

NOVETNY, V., Basset Y., MILLER, S.E., WEIBLEIN, G. D., BREMER, B, CIZEK, L. & DRoZD, P. (2002) Low host specificity of herbivorus inscets in a tropical forest. NATURE 416

NOWAK, V., PERSIJN, D., RITTENSCHOBER, D. & CHARRONDIERE, U. R. (2016) Review of food composition data for edible insects. Food Chemistry 193, 39-46

OCHOA SANABRIA, C., HOGAN, N., MADDER, K., GILLOTT, C., BLAKLEY, B., REANEY, M., BEATTIE, A. & BUCHANAN, F. (2019) Yellow mealworm larvae (Tenebrio molitor) fed mycotoxin-contaminated wheat-A possible safe, sustainable protein source for animal feed? Toxins (Basel) 11(5), 282

OHTA, M., ISHII, K. & UENO, Y. (1977) Metabolism of Trichothecene mycotoxins: I. Microsomal deacetylation of T-2 toxin in animal tissues. The Journal of Biochemistry 82, 1591-1598

OONINCX, D. & DE BOER, I. (2012) Environmental impact of the production of mealworms as a protein source for humans–A life cycle assessment. PLoS ONE

OONINCX, D. G., VAN ITTERBEECK, J., HEETKAMP, M. J., VAN DEN BRAND, H., VAN LOON, J. J. & VAN HUIS, A. (2010) An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. PLOS One 5

PAN, D., MIONETTO, A., TISCORNIA, S. & BETTUCCI, L. (2015) Endophytic bacteria from wheat grain as biocontrol agents of Fusarium graminearum and deoxynivalenol production in wheat. Mycotoxin Research 31, 137-143

PANAGIOTAKOPULU, E. (2001) New records for ancient pests: archaeoentomology in Egypt. Journal of Archaeological Science 28, 1235-1246

PEREIRA, V., FERNANDES, J. & CUNHA, S. (2015) Comparative assessment of three cleanup procedures after QuEChERS extraction for determination of trichothecenes (type A and type B) in processed cereal-based baby foods by GC–MS. Food Chemistry 182, 143-149

PFEIFFER, R. L., SWANSON, S. P. & BUCK, W. B. (1988) Metabolism of T-2 toxin in rats: effects of dose, route, and time. Journal of Agricultural and Food Chemistry 36, 1227-1232

POMA, G., CUYKX, M., AMATO, E., CALAPRICE, C., FOCANT, J. F. & COVACI, A. (2017) Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. Food Chem Toxicol 100, 70-79

PUNZO, F. (1975) Effects of temperature, moisture and thermal acclimation on the biology of Tenebrio molitor (Coleoptera: Tenebrionidae). Retrospective Theses and Dissertations, Iowa State University

RADOVÁ, Z., HOLADOVÁ, K. & HAJŠLOVÁ, J. (1998) Comparison of two clean-up principles for determination of trichothecenes in grain extract. Journal of Chromatography A 829, 259-267

RAHEEM, D., CARRASCOSA, C., OLUWOLE, O. B., NIEUWLAND, M., SARAIVA, A., MILLAN, R. & RAPOSO, A. (2019) Traditional consumption of and rearing edible insects in Africa, Asia and Europe. Critical Reviews in Food Science and Nutrition 59, 2169-2188

RAMOS-ELORDUY, J. (2006) Threatened edible insects in Hidalgo, Mexico and some measures to preserve them. Journal of Ethnobiology and Ethnomedicine 2, 51

RAUCH, N. & NAUEN, R. (2003) Identification of biochemical markers linked to neonicotinoid cross resistance in Bemisia tabaci (Hemiptera: Aleyrodidae). Archives of Insect Biochemistry and Physiology: Published in Collaboration with the Entomological Society of America 54, 165-176

SCHOLLENBERGER, M., LAUBER, U., JARA, H. T., SUCHY, S., DROCHNER, W. & MÜLLER, H.-M. (1998) Determination of eight trichothecenes by gas chromatography–mass spectrometry after sample clean-up by a two-stage solid-phase extraction. Journal of Chromatography A 815, 123-132

SCHOTHORST, R. C. & VAN EGMOND, H. P. (2004) Report from SCOOP task 3.2. 10 "collection of occurrence data of Fusarium toxins in food and assessment of dietary intake by the population of EU member states": Subtask: trichothecenes. Toxicology letters 153, 133-143

SHIFRIN, V. I. & ANDERSON, P. (1999) Trichothecene mycotoxins trigger a ribotoxic stress response that activates c-Jun N-terminal kinase and p38 Mitogen-activated protein kinase and induces apoptosis. Journal of Biological Chemistry 274

SOCHA, R. & SEHNAL, F. (1972) Inhibition of adult development in Tenebrio molitor by insect hormones and antibiotics. Journal of Insect Physiology 18, 317-337

SOKOLOVIĆ, M. & ŠIMPRAGA, B. (2006) Survey of trichothecene mycotoxins in grains and animal feed in Croatia by thin layer chromatography. Food Control 17, 733-740

STORK, N. E. (2018) How many species of insects and other terrestrial arthropods are there on earth? Annual Review of Entomology 63, 31-45

TAN, H. S. G., FISCHER, A. R. H., TINCHAN, P., STIEGER, M., STEENBEKKERS, L. P. A. & VAN TRIJP, H. C. M. (2015) Insects as food: exploring cultural exposure and individual experience as determinants of acceptance. Food Quality and Preference 42, 78-89

TAN, H. S. G., FISCHER, A. R. H., VAN TRIJP, H. C. M. & STIEGER, M. (2016) Tasty but nasty? Exploring the role of sensory-liking and food appropriateness in the willingness to eat unusual novel foods like insects. Food Quality and Preference 48, 293-302

THOMPSON, W. & WANNEMACHER JR, R. (1990) In vivo effects of T-2 mycotoxin on synthesis of proteins and DNA in rat tissues. Toxicology and Applied Pharmacology 105, 483-491

THRANE, U., ADLER, A., CLASEN, P. E., GALVANO, F., LANGSETH, W., LEW, H., LOGRIECO, A., NIELSEN, K. F. & RITIENI, A. (2004) Diversity in metabolite production by Fusarium langsethiae, Fusarium poae, and Fusarium sporotrichioides. International Journal of Food Microbiology 95, 257-266

THUVANDER, A., WIKMAN, C. & GADHASSON, I. (1999) In vitro exposure of human lymphocytes to trichothecenes: individual variation in sensitivity and effects of combined exposure on lymphocyte function. Food and Chemical Toxicology 37, 639-648

TOMBERLIN, J. K. & VAN HUIS, A. (2020) Black soldier fly from pest to 'crown jewel' of the insects as feed industry: an historical perspective. Journal of Insects as Food and Feed 6, 1-4

TOMBERLIN, J. K., VAN HUIS, A. & BENBOW, M. E., et al. (2015) Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. Journal of Insects as Food and Feed 1, 307-309

TORP, M. & NIRENBERG, H. I. (2004) Fusarium langsethiae sp. nov. on cereals in Europe. International journal of food microbiology 95, 247-256

UENO, Y. (1984) Toxicological features of T-2 toxin and related trichothecenes. Fundamental and applied Toxicology 4, 124-132

VAN BROEKHOVEN, S., GUTIERREZ, J., DE RIJK, T. C., DE NIJS, W. C. M. & VAN LOON, J. J. A. (2017) Degradation and excretion of the Fusarium toxin deoxynivalenol by an edible insect, the yellow mealworm (Tenebrio molitor L.). World Mycotoxin Journal 10, 163-169

VAN HUIS, A., DICKE, M. & VAN LOON, J. J. A. (2015) Insects to feed the world. Journal of Insects as Food and Feed 1, 3-5

VAN HUIS, A., VAN ITTERBEECK, J., KLUNDER, H., MERTENS, E., HALLORAN, A., MUIR, G. & VANTOMME, P. (2013) Edible insects: future prospects for food and feed security, http://www.fao.org/3/i3253e/i3253e.pdf. Accessed: 22.09.2020

WACHOWSKA, U. & GŁOWACKA, K. (2014) Antagonistic interactions between Aureobasidium pullulans and Fusarium culmorum, a fungal pathogen of winter wheat. BioControl 59, 635-645

WACHOWSKA, U., PACKA, D. & WIWART, M. (2017) Microbial inhibition of Fusarium pathogens and biological modification of trichothecenes in cereal grains. Toxins 9, 408

WESTHEIDE, W. & RIEGER, R. (1996) Spezielle Zoologie. 1. Einzeller und Wirbellose Tiere. Heidelberg, Spektrum, Akademischer Verlag

YANG, Y., YANG, J., WU, W. M., ZHAO, J., SONG, Y., GAO, L., YANG, R. & JIANG, L. (2015a) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 1. Chemical and physical characterization and isotopic tests. Environmetnal Science & Technology 49, 12080-12086

YANG, Y., YANG, J., WU, W. M., ZHAO, J., SONG, Y., GAO, L., YANG, R. & JIANG, L. (2015b) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: Part 2. Role of gut microorganisms. Environmetnal Science & Technology 49, 12087-12093

YEN, A. L. (2009) Edible insects: traditional knowledge or western phobia? Entomological Research 39, 289-298

YEN, A. L. (2015) Insects as food and feed in the Asia Pacific region: current perspectives and future directions. Journal of Insects as Food and Feed 1, 33-55

YOSHIZAWA, T., OKAMOTO, K., SAKAMOTO, T. & KUWAMURA, K. (1985) In vivo metabolism of T-2 toxin, a trichothecene mycotoxin. On the formation of depoxydation products. JSM Mycotoxins 1985, 9-12

ZHANG, Z. Q. (2011) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Auckland, Magnolia Press

ZIELIŃSKA, E., BARANIAK, B., KARAŚ, M., RYBCZYŃSKA, K. & JAKUBCZYK, A. (2015) Selected species of edible insects as a source of nutrient composition. Food Research International 77, 460-466

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