Literature review for risk assessment of insects

Advisory report from DCA – National Center for Food and Agriculture

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Data sheet

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1 Preface

The Danish production of protein is limited, causing a need for importing large amounts of plant-based proteins. However, farmed insects provide an alternative source of protein in feedstuff for livestock, which can help to meet the increasing demands of proteins worldwide and limit the negative impact from import of soya. Currently, rearing of insects in EU is constrained by the legislation requirements of using feed-grade substrates only, but the economic feasibility and not least sustainability could be improved significantly by allowing by-products and food residues that for now are considered as waste or unsafe. This includes kitchen and food waste, former foodstuff of vegetable origin which is spoiled by e.g., molds and/or contains packaging materials as well as former foodstuff with animal content (fish or meat). However, it is essential that any substrate used for rearing of insects does not pose a risk for the insect themselves, animals and humans feeding on insects as well as the environment.

The purpose of this literature review is to collect information on the existing knowledge on the safety of rearing insects for food and feed if various none-feed-grade organic materials would be applied as substrates for the insects. The review shall help to identify gaps regarding the research needed to document that certain none-feed grade by-products and residues can be applied safely as substrate for insects without posing a risk and thereby open for relaxation of the current EU regulations on feed for insects.

The hazards addressed in this review are based on the EFSA report from 2015 "Risk profile related to production and consumption of insects as food and feed" and a memorandum from the DTU Food Institute (Fareprofil for substrater til insektopdræt; Danger profile for substrates for insect breeding, 2018, in Danish only). The current review is based on recent scientific papers published after the 2018 memorandum, and include papers found in a literature search from 2021 (Jensen *et al*. 2022) and a new literature search made in 2022.

Based on the literature review, a research plan has been made to pinpoint which knowledge gabs should be further addressed, to be able to assess whether it is safe to feed insects with kitchen food waste, former food, and residual products with packaging residues, including spoiled feed. Even though studies have been made on transfer and accumulation of the hazards mentioned in the reviews, more studies are needed on several compounds e.g., pesticides, PFAS and compounds in food contact materials. Moreover, research is needed on the substrate in relation to applicability (volume and suitability for the insect), characterization of the microbiological quality, effect of storage conditions and effect of different treatment methods. In addition, more research is needed in relation to the treatment of insects to control microbial load.

Content

2 Dansk sammendrag

Insekter som proteinkilde til produktionsdyr ses som et spirende potentielt alternativ til konventionelle foderproteiner, som kun produceres i begrænset omfang i Danmark og derfor må importeres i store mængder. Øget produktion af insektprotein til foderbrug vil hjælpe med at reducere behovet for importerede planteproteiner, og de deraf følgende negative effekter som fældning af regnskov for at producere sojabønner eller overfiskning for at imødekomme behovet for fiskemel. Insekter har desuden en meget anderledes biologi end traditionelle produktionsdyr og kan potentielt vokse på organiske materialer der ellers anses for at være affald. Ved udnyttelse af sådanne affaldsprodukter vil produktionen af insekter kunne foregå uden at konkurrere med fodermidler til andre fødevareproducerende dyr. Betingelsen for at kunne opdrætte insekter på sådanne affaldsprodukter vil være, at de ikke vil udgøre en risiko for insekterne selv, for de dyr, som fodres med insekter, eller for mennesker, der anvender insekter til fødevarer. Foderlovgivningen er ikke skrevet med fokus på fodring af insekter. Med henblik på at understøtte fremtidige ændringer af lovgivningen for at sikre større bæredygtighed mht. udnyttelse af restprodukter til ressourceeffektiv insektproduktion, er det relevant at undersøge, om køkken- og madaffald eller andre fødevarerester som fx tidligere fødevarer, der ikke kan anvendes lovligt i dag, fx fordi de er fordærvede, angrebet af mug, potentielt mikrobielt forurenet eller indeholder emballagerester kan anvendes sikkert som foder til insekter, og om insekterne, der er fodret med disse produkter, uden risiko kan anvendes til enten foder eller fødevarer.

Formålet med denne litteraturgennemgang er at undersøge det nuværende forskningsmæssige grundlag, og derefter lave en oversigt over den supplerende forskning, der skal til for at vurdere, om man sikkert vil kunne lempe EU-lovgivningen og dermed øge ressourceudnyttelsen ved at anvende nye restprodukter som foder til insekter.

De farer, der er dækket af denne litteraturgennemgang, er baseret på EFSA-rapporten fra 2015 "Risk profile related to production and consumption of insects as food and feed " og et notat fra DTU Fødevareinstituttet (Fareprofil for substrater til insekt opdræt, 2018, kun på dansk). Den aktuelle gennemgang er hovedsageligt baseret på nyere videnskabelige artikler offentliggjort efter 2018, herunder artikler fundet i en litteratursøgning fra 2021 (Jensen *et al*. 2022) og en ny litteratursøgning foretaget i 2022.

På grundlag af litteraturgennemgangen er der udarbejdet en forskningsplan, der adresserer den viden der mangler, for at være i stand til at vurdere, om det er sikkert at give mulighed for at fodre insekter med fordærvet og/eller frisk køkken- og madaffald, tidligere fødevarer og restprodukter med emballagerester.

Selvom der er lavet studier om overførsel og akkumulering af de komponenter der er nævnt i litteraturgennemgangen, er der behov for flere undersøgelser på fx pesticider, PFA'er og forbindelser i fødevarekontaktmaterialer. Desuden er der behov for undersøgelse af potentielle restprodukter i forhold til anvendelighed (volumen og egnethed for insektet), karakterisering af produkternes mikrobiologiske kvalitet, effekt af deres opbevaringsforhold og forskellige behandlingsmetoder. Derudover er der behov for mere forskning i efterbehandling af insekter for at kontrollere den mikrobielle belastning.

3 Introduction

3.1 Background and method

The purpose of this review is to collect information and identify gaps in the existing literature on the safety of insects as food and feed if reared on by-products and residues from food production, which are currently not allowed according to EU feed law. The by-products of interest as potential substrates for rearing of edible insects is 1) Former Foodstuff (FF) as cakes and bread that are spoiled but otherwise legal, 2) FF or by-products containing food packaging materials e.g., plastic, paper and cardboard, 3) FF with meat and fish (i.e. animal content besides processed eggs, milk and honey) both fresh and spoiled, 4) Kitchen- and food waste (fresh or spoiled) of vegetable and animal origin e.g., from commercial kitchens and canteens.

The review focuses on the eight species of insects that are currently allowed for feed and in the form they are commonly used; these are larvae of beetles, butterflies, or flies, and the adult individuals of grasshoppers and crickets. The eight species allowed for feed are: Black Soldier Fly (*Hermetia illucens*), Common Housefly (*Musca domestica*), Yellow Mealworm (*Tenebrio molitor*), Lesser Mealworm (*Alphitobius diaperinus*), House cricket (*Acheta domesticus*), Banded cricket (*Gryllodes sigillatus*), Field Cricket (*Gryllus assimilis*) and silkworm (*Bombyx mori*) (i.e., Regulation (EU) 2021/1925).

The hazards covered in this review are based on the EFSA report from 2015 "Risk profile related to production and consumption of insects as food and feed" and a memorandum from the DTU Food Institute (Fareprofil for substrater til insektopdræt; Danger profile for substrates for insect breeding, 2018, in Danish only). The memorandum from DTU was based on the EFSA publication but with updated literature. In 2021, prior to a series of experiments, a literature search was performed to identify available literature on hazards related to feed safety on *Hermetia illucens* larvae (black soldier fly larvae; BSFL) and *Tenebrio molitor* larvae (common or yellow mealworm) (Jensen *et al*. 2022). The current review is based on recent scientific papers mainly published after the 2018 memorandum, and include papers found in a literature search from 2021 (Jensen *et al*. 2022) and a new literature search made in 2022. The search has been made in open literature from 2018 to 2022, both years included, using Scopus and supplemented by data from Danish monitoring programs. Based on a comparative search in the data bases Scopus, PubMed and Web of Science it has been concluded that the literature for this review can be covered by searching in Scopus. Most literature is found on BSFL and yellow mealworm but when relevant, the hazards are described in other of the eight species allowed for feed e.g., the house cricket.

3.1.1 Feed legislation

Feed for farmed animals including insects must comply with the provisions of the EU feed legislation in EU, which is made up of a series of regulations and directives. The Marketing and Use of Feed Regulation stipulates that feed must be safe and has no direct adverse effect on the environment or animal welfare (EU No. 767/2009) and prohibits materials like faeces, urine, sewage sludge and packaging materials. Immediately approved feed of vegetable origin is listed in the Regulation on the Catalogue of feed materials (EU No. 68/2013, and amendment EU No. 2017/1017) and supplemented by the EU register of feed additives (EC No. 1831/2003). Contrary, the usage of products of animal origin as feed is strictly regulated by the regulations on Animal By-Products and derived products (EU No. 1069/2009 & implementing regulation EU No, 142/2011). Further, the TSE regulation (transmissible spongiform encephalopathy) (EC No. 999/2001 and amendments to annex in EU 2017/893 EU 2021/1372), stipulates, which animals can be fed with feed of animal origin like insects, and bans specifically the use of these products in feed for ruminants. Moreover, it is not allowed to use any products originating from one species as feed for the same species (ban of cannibalism).

The description here on regulations and directives is not exhaustive. For further information regarding rules for farmed insects see the overview by the Danish Veterinary and Food Administration (DVFA).

3.1.1.1 Rules for using animal products as feed

Products that are not allowed:

Animal by-products are divided into three categories depending on risks involved, where category 3 materials constitute the lowest risk and is the only category potentially allowed in feed. Category 1 includes materials posing the greatest risk regarding spread of transmissible spongiform encephalopathies (TSE) such as mad cow disease (BSE). Category 2-materials include livestock manure, cadavers of animals that died by themselves, diseased animals and everything that is neither category 1- or 3-material. A requirement for the use of category 3-materials in feed is processing by specifications given in Annex X of the Implementing Regulation (EU No 142/2011) resulting in 'Processed Animal Protein (PAP). However, irrespective of processing, category 3-materials like kitchen and catering waste as well as former foodstuffs with animal contents are prohibited in feed for food-producing animals incl. insects. Excepted are former foodstuffs containing milk, egg, honey, fat, collagen, and gelatine, as long as they are processed as food while e.g., dough with raw egg is prohibited.

Products from animals that are allowed as feed:

Animal by-products specified as category-3 materials constitute the lowest risk and are divided into two groups based on origin – either from slaughterhouses or of other origin. There are specific requirements for processing of different types of category 3-material (Annex X in the Implementing Regulation (EU No. 142/2011). Sterilization by pressure constitutes the most stringent treatment and by-products from mammals must undergo this treatment, whereas alternative methods may be applied for other products if the requirements for the end product are met. These are absence of salmonella in 25 g and Enterobacteria levels ≤ 300 CFU/g (and maximum 2 of 5 samples having between 10 and 300 CFU/g). The products of animal origin allowed in feed for insects if processed according to the specifications for animal by-products entail rendered fat, fish oil, eggs, milk, blood-products of none-ruminants, hydrolysed protein and gelatine and collagen of none-ruminants and fishmeal.

4 Biological hazards

The biological hazards included in this review mainly include the zoonotic bacteria and spore forming bacteria involved in food poisoning. The severe animal disease viruses, prions and parasites are also shortly addressed, but in relation to edible insects, only limited new literature has been published since the EFSA risk profile and the DTU memorandum from 2018. The review describes the transfer of microorganisms from substrate to the insects and to the degree possible also the fate of microorganisms post-harvest depending on processing. The review focuses on individual studies and does not cover review papers. There are several relevant reviews (Cappelli *et al.* 2020; Garofalo *et al.* 2019; van der Fels-Klerx *et al.* 2018; Vandeweyer *et al.* 2021) focusing on microbiological hazards in edibles insect.
Zoonotic infectious agents from especially unprocessed food waste or former foodstuff of animal origin include human pathogens like *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica* and *Staphylococcus aureus* (MRSA). Additionally, there exists a range of severe notifiable livestock diseases (BEK no 1191, 24/08/2022). However, in Denmark the occurrence of these diseases is very rare as it is the case for e.g., foot-and-mouth disease, African and classical swine fever (ASF/CSF), and Aujeszky's disease. However, in recent years, African swine fever has been occurring in several European countries including Germany, while Highly Pathogenic Avian influenza (HPAI) has been recurring in Denmark (EFSA 2019; Sauter-Louis *et al*. 2021). The significance of different indirect transmission routes has been under investigation but is not fully resolved (Fischer *et al*. 2020; Olesen et. al. 2020). Long-term survival of ASF virus in various pig tissues implies a risk of contamination from untreated meat, while processing like heating may inactivate the virus (Olesen *et al*. 2020). Although DNA of ASF virus was detected in heat-treated field crops (1 h 7°5C), it was not infectious (Fischer *et al*. 2020). There are to our knowledge no new studies on these livestock diseases in relation to edible insects as the focus mainly is on the role of insects as vectors for transmission (Bonnet *et al*. 2020).

Former foodstuff and food waste may entail an enhanced risk of spoilage of particularly perishable food depending on the time and storage conditions along the way from collection until the use as substrate for insects. Depending on the composition of the waste in terms of nutrients, available water (water activity, aw), pH and salt content, the food waste may support the growth of harmful bacteria and or moulds. Certain moulds like Aspergillus, Penicillium and Fusarium can produce different types of mycotoxins, which are addressed under the chemical hazards.

Another issue is the emergence of SARS-CoV-2. However, insects are expected to constitute a very low risk as they lack a receptor that can bind SARS-CoV-2, and coronaviruses have never been recorded in insect microbiomes (Dicke *et al*. 2020).

4.1 Prions

Prion diseases or TSEs (transmissible spongiform encephalopathy) mainly concern ruminants, and primarily BSE (bovine spongiform encephalopathy) constitutes a risk of transmission between animal species as it may lead to the variant Creutzfeldt-Jacob disease (vCDJ) in humans. The main reservoirs of prions are brain, spinal cord, eyes, tonsils and parts of the intestines and the practice of removing these specified risk materials (SRM) reduces the risk of prions to occur substantially. If present, prions are extremely resistant to standard inactivation methods including the most stringent processing method required under EU legislation for processed animal proteins (EC no. 142/2011) i.e., 133°C for 20 min at a pressure of 3 bar. Preventing introduction of prions via substrate is therefore the key to control, as it cannot be excluded entirely that insects can act as passive vectors of prions.

Although the occurrence of BSE in EU now is rare (EFSA, 2018), the concerns related to the recycling of BSE back into the food chain gives rise to similar concerns for rearing insects on animal content if the insects are fed to animals of the same species, in the following referred to as a 'two-species feedback loop'. However, the actual risk is very uncertain, and means to deduce the source of animal feed proteins will be necessary in order to assess if the loop has been avoided (Niedzwiecka *et al*. 2019). In one study, bovine DNA in black soldier fly larvae (BSFL) fed with spiked substrate was detected (Belghit *et al*. 2021). However, a previous study carried out by the authors of this review, did not find DNA from ruminants or chicken in BSFL fed with spiked substrate (Jensen *et al*. 2022), but found one sample of BSFL with porcine DNA.

4.2 Parasites

Intestinal parasites like *Cryptosporidium* spp., *Giardia* and *Echinococcus* excreted by animals may contaminate fruit and vegetables via contaminated water, but although the occurrence is unknown, food borne infections are rare. Other parasites like *Trichinella* spp. are related to raw (non-frozen) fish (anisakid nematodes) and raw meat. Danish pigs are free of this parasite, but the risk of *Trichinella spiralis* is however increased when pigs have access to outdoor areas.

Another parasite, *Toxoplasma gondii* causing toxoplasmosis in humans, is transmitted directly via resistant oocysts (eggs) excreted by infested cats or indirectly via oocysts spread in the environment leading to contamination of e.g., vegetables. Furthermore, tissue cysts can be found in muscle tissue of raw pig, lamb, and game meat. Based on antibodies, an apparent seroprevalence of 2% was found in Danish indoor-raised finishers, while the prevalence in outdoor-raised finishers was 11% (Anon. 2022). The tissue cysts are destroyed when meat is heated above 67°C, smoked, salted or frozen (-20°C) for at least 3 days. The oocyst is otherwise resistant (Mirza Alizadeh *et al*. 2018) and a key to control is via hygienic management in rearing of insects (access restriction to cats) (Percipalle *et al*. 2021). The presence of *Toxoplasma gondii* was investigated in 16 different insect foods and was detected in one consisting of dehydrated mealworms (Percipalle *et al*. 2021).

Generally, data on occurrence of parasites in food is sparse, including to which degree edible insects will take up parasites if present in their substrate. Müller *et al*. (2019) found that few *Eimeria* oocysts and eggs of *Ascaris suum* could be found in the intestines of BSFL after 10 days of feeding on contaminated substrate (200.000 eggs / g), and the oocyst or egg walls seemed not to be deteriorated by the BSFL.

4.3 Bacteria:

4.3.1 Campylobacter

Campylobacter jejuni is the predominant cause of foodborne infections in humans and is primarily transferred by fresh and undercooked meat, especially chicken meat. *Campylobacter* prevalence in poultry varies depending on production method and source. The occurrence of *Campylobacter* in conventional vs. organic broiler meat is 22.2% / 36.5% in slaughterhouse samples, 11.9% / 30.4% in Danish retail and 64.3% / 69.4% in imported meat (Anon. 2022).

Especially former foodstuff and food waste with meat (particularly poultry) content that has not been heat treated or processed to eliminate *Campylobacter* comprise a risk of transferring *Campylobacter*. Most insect studies focusing on *Campylobacter* concern the potential of especially flies as transmission vectors. Nevertheless, a microbiota composition study in Kenya by Tanga (2021) found a high abundance of *Campylobacter* spp. in BSFL fed 14 d on food waste, poultry or rabbit manure and even spent grains from a brewery. In another BSFL study, the *Campylobacter* spp. level in BSFL increased with rearing temperature, from 3.2 log CFU/g at 20°C to 4.7 log CFU/g at 33°C, respectively, although no *Campylobacter* was detected in the vegetable-based substrate (Raimondi *et al*. 2020). Proliferation of *Campylo-*

bacter usually requires a host, which BSFL may have constituted, but in other studies where C*ampylo-bacter* was ingested by houseflies or larvae, the *Campylobacter* bacteria seemed to be cleared within few days or less depending on dose (Nordentoft *et al*. 2017; Jensen unpublished; Skovgaard *et al*. 2011).

4.3.2 Salmonella

The prevalence of *Salmonella* in Danish pork and beef meat samples is generally very low (<1%) and for broiler meat there is a zero tolerance for the presence of *Salmonella*. However, 12-18% of batches of Danish pork and imported pork and broiler meat test *Salmonella* positive (DVFA 2019). Danish eggs are very rarely positive and imported eggs are constrained by *Salmonella* status requirements.
Especially former foodstuff and food waste with animal content that has not been heat treated or processed to eliminate *Salmonella* comprise a risk of transferring *Salmonella*, although contamination of other foods can occur. *Salmonella* was detected in BSFL and *Acheta domesticus* or house cricket reared on combinations of brewer's and kitchen waste in Kenya (Nyangena *et al*. 2020). Other than that, absence of *Salmonella* is mostly reported for reared insects for food and feed except a few cases, probably due to the use of feed grade substates (Kashiri *et al*. 2018; Raimondi *et al*. 2020). Nonetheless, the study by Raimondi *et al*. (2020) showed that although the substrate was free of *Salmonella*, BSFL tested positive and more often when the rearing temperature was 33°C vs. 20 or 27 degrees.
However, *Salmonella* may be introduced via contaminated substrate or environment and four studies have investigated the fate of introduced *Salmonella* in spiking studies with Tm and BSFL.
For BSFL, the effect of exposure dose (3-7 log CFU / g substrate) was assessed, and one study indicated an increase in the *Salmonella* level in the BSFL over the study period of 6 days (De Smet *et al*. 2018). However, a marked difference between replicate experiments and not least detection of *Salmonella* in the controls (non-spiked), which indicate cross-contamination, undermines plausible conclusions. In another study at an initial contamination level of 7 CFU / g in the substrate, the level in BSFL decreased from 4.0 log CFU / g at day 3 to 2.0 log CFU / g at day 17 (Grisendi *et al*. 2022). In the substrate with BSFL, the *Salmonella* level was reduced to 3.7 log CFU / g at end of study and was significantly lower than the level in control substrate without larvae except on day 13. This indicated that BSFL exerted some sanitizing effect against *Salmonella* although not eliminated completely, as also previously reported in studies on waste conversion by larvae (Lopes *et al*. 2020; Zhang *et al*. 2021). However, the reduction capacity seems to depend on the bacteria species as *Enterococcus* spp. levels were found to increase 1 log (Lopes *et al*. 2020) and *E. coli* declined 2-3 log while *Listeria monocytogenes* levels remained constant over 8 days (Swinscoe *et al*. 2020).

For *Tenebrio molitor* or yellow mealworm, there seems to be a clear relationship between the exposure dose in the substrate and the level found in the larvae (Jensen *et al*. 2020; Wynants *et al*. 2019a). A contamination level of 2 log CFU / g substrate only resulted in positive larvae shortly after spiking at start of the experiment, and the substrate was *Salmonella*-negative within the first week (Jensen *et al*. 2020). At 4 log CFU / g substrate, the *Salmonella* level in larvae and substrate was low, but both remained culture-positive following enrichment until the end of study on day 14. Only at the highest contamination level of 7 log CFU / g substrate, *Salmonella* was detectable on day 14 without enrichment in both larvae (1.9 log CFU / g) and substrate (3.7 log CFU / g). Similarly, Wynants *et al* (2019a) found 4.1 log CFU / g in larvae on day 7 after contamination with 7 log CFU/g substrate. At a contamination level of 2 log CFU / g substrate, 11 of 18 (not disinfected) or 5 of 11 (disinfected) larvae samples were still positive on day 7. Generally, over the time course of both studies, the substrate showed higher levels of *Salmonella* than larvae. Further, the ethanol disinfected larvae were less often positive indicating that some *Salmonella* remain on the outside rather than being ingested.

4.3.3 Listeria

Listeria monocytogenes (*L. m*.) is ubiquitous, present in soil, plants, sewage, animal guts, and particularly capable of hiding in food production environments and grows at low temperatures (standard refrigerator). The main sources of contamination are meat toppings, cold-smoked fish, ready meals insufficiently re-heated, raw cheeses and unpasteurized milk. Generally, *L. monocytogenes* levels in ready-to-eat products should be kept < 100 CFU / g to ensure food safety within the shelf life of products. Food residues to be allocated into insect substrate are likely to have exceeded their 'normal' shelf-life as food and since *Listeria* bacteria at the same time are able to grow even under refrigerater temperature, this may infer an increasing *Listeria* contamination risk over time, if conditions are otherwise favourable (e.g., appropriate water activity (A_w) and salt content).

Listeria monocytogenes is mostly reported as absent in reared insects for food and feed*,* but the presence of unspecified *Listeria* spp. in BSFL in levels as high as 7.5 and 5.2 log CFU / g was reported by Saucier *et al*. (2022) and Larouche *et al*. (2019), respectively. Raimondi *et al*. (2020) found that the level of *Listeria* spp. in BSFL was higher when reared at 33°C (5.8 log CFU / g) compared to 20°C (4.8 log CFU / g) and higher than the level found in the vegetable-based substrate (2.6 log CFU ±0.4).

In a BSFL spiking study with seaweed contaminated with 8 log CFU /g *L. monocytogenes*, the presence of *L. m.* in larvae ranged from 5.5 to 6.5 log CFU / g within the 8 days of study, while no clear decline was

seen in the *L. m.* level in the substrate (Swinscoe *et al*. 2020). Contrary, Grisendi *et al*. (2022) found a decrease of *L.m*. in both substrate (from 7.24 to 5.93 log CFU / g) and BSFL (from 6.56. to 4.45 log CFU / g) over a period of 17 days. Moreover, the *L. m.* level in substrate without larvae did not decrease over time indicating a sanitizing effect of the larvae.

In a yellow mealworm spiking study with 1-7 log CFU / g *Listeria innocua* in the substrate, bacteria level in yellow mealworm seemed to range from 3.78 to 6.34 log CFU / g over the 7 days of study irrespective of the initial inoculum, indicating that even low contamination levels infer a risk of *Listeria* positive larvae (Bellegia *et al*. 2020). In another spiking study with *L. monocytogenes* (8 log CFU / g), the larvae seemed to support a high *L. m*. in the substrate over the 7 days of study (5.3- 6.6 log CFU /g) opposed to substrate without larvae (4.9 log CFU / g on day 7), while an insignificant increase was observed in the larvae (from 3.6 to 4.6 log CFU / g) (Mancini *et al*. 2019b).

4.3.4 Escherichia coli

Escherichia coli belongs to the Enterobacteriaceae family and comprises both non-pathogenic and pathogenic strains, and generally indicates faecal contamination and the level of hygiene. Microbiological criteria are set for the accepted level in different food commodities e.g., maximum 500 CFU / g for minced meat and maximum 1000 CFU / g for pre-cut fruit and vegetables (EC No. 2073/2005). For insects it is often just the overall level of Enterobacteriaceae that is reported rather than *E. coli* specifically, and for derived products for feed use (EC No. 142/2001) the Enterobacteriaceae limit is 300 CFU /g. However, *E. coli* levels of 4.5 log CFU / g (Bessa *et al*. 2021) and 7.5 log CFU / g (Saucier *et al*. 2022) have been reported for BSFL. Spiking experiments with high levels (9-10 log CFU /g) of two different strains of *E. coli* resulted in high levels in the BSFL (7-8 log CFU / larvae), which declined 2-3 log over the 8 days of study. In house crickets, a level of 6.7 log CFU / g *E. coli* was reported by Fröhling *et al*. (2020).

4.3.5 Yersinia entorocolitica

Yersinia enterocolitica is also entailed in the Enterobacteriaceae family and undercooked pork meat is the main infection route although the prevalence in pigs is not routinely monitored. Like other intestinal pathogens excreted in faeces, *Y. enterocolitica* may also contaminate vegetables if they are exposed to faeces directly or indirectly via contaminated water. Reports regarding occurrence of *Yersinia* in edible insects seem to be lacking.

4.3.6 Staphylococcus aureus

Staphylococcus aureus can be present on skin and mucuos membranes in both humans and animals including methicillin resistant *Staphylococcus aureus* (MRSA) (animal type), which have been found in pork meat (DVFA 2016). However, most cases of *S. aureus* food poisoning are due to contamination after heat treatment of foods followed by insufficient cooling that support growth of *S. aureus* and then possible production of toxins. Several studies report findings of coagulase positive staphylococci (CPS) implying the presence of *S. aureus* but not exclusively (Borremans *et al*. 2018; Osimani *et al.*, 2018; Bawa *et al*. 2019; Nyangena *et al*. 2020; Mancini *et al*. 2019ac, 2022; Li *et al*. 2020; Raimondi *et al*. 2020; Jucker *et al*. 2021). The levels seem to vary from below detection level to 8 log CFU / g. A single spiking experiment showed that *S. aureus* in BSFL remained below the detection level (< 2 log CFU /g) for the 6 days of study even at a contamination level of 7 log CFU / g (Gorrens *et al*. 2021). Further, the *S. aureus* level in the substrate declined faster when larvae were present indicating a sanitizing effect.

4.3.7 Spore forming bacteria

The spore forming bacteria *Bacillus cereus* and *Clostridium perfringens*, and more rarely *Clostridium botulinum,* can cause food poisoning by production of toxins when present in high numbers. The formation of very resistant endospores enables the bacteria to survive during heat treatment that normally eliminate vegetative bacteria. When growth conditions with respect to level of oxygen, water activity, temperature, pH (>4) and salt content, are favourable, spores return into the vegetative state and produce toxins.

B. cereus is naturally occurring in soil, plants and in water and typically enters the food chain via contaminated spices, milk products, eggs, vegetables and starch rich products and ready-to-eat food. *B. cereus* can produce two types of toxins, and noticeably, the emetic toxin type is produced within the food, and once produced very resistant to following heat treatments as it can persist about 90 min at 126°C. As it usually requires vegetative cell numbers to reach 5-6 log CFU / g before toxin production occurs, growth prevention via exposure to unfavourable conditions is a key to avoid the toxins.

Likewise, *C. perfringens* occurs in the environment and contaminates foods via direct or indirect contact with soil or water. Typical sources are meat or fish and products thereof, fruits, vegetables, honey, and spices. Again, high cell levels (> 5 log CFU / g) are required before toxin production occurs.

For BSFL, endospore levels between 2.2 to 8 log CFU / g have been reported and more often with higher levels in the larvae compared with the level in the substrate (Larouche *et al*. 2019; Wynants *et al* 2019b; Raimondi *et al* 2020; Osimani *et al*. 2021). Often the present endospores are not speciated further, however, Raimondi also reported 2.3 log CFU / g *B. cereus* in the larvae while the substrate tested negative.

In yellow mealworm, the reported endospore levels were from <1 to 4.4 log CFU / g and Fasolato *et al*. (2018) reported levels of <2 to 3 log CFU / g bacteria of the *Bacillus cereus* group (gr.) in shelf-stable food products (a_w <0.6). However, the group of *B. cereus* also comprises harmless species closely related to *B. cereus*.

In house crickets, the levels of endospores vary considerably from <1 to 8.1 log CFU / g (Fasolato *et al*. 2018). Jucker *et al*. (2021) also found bacteria belonging to the *B. cereus* gr. (3.2 log CFU / g) in substrate for house crickets consisting of 'laying hen diet' but without finding *B. cereus* gr.in house crickets (<1 log CFU / g). Other studies found *B. cereus* gr. in cricket meal and various cricket food products in levels ranging from <1 to 5 log CFU / g (Fasolato *et al*. 2018; Machado *et al*. 2020; Frentzel *et al*. 2022;) and 4.2 log CFU / g in frozen crickets (Bawa *et al*. 2019).

In silkworms, the reported levels of *B. cereus* gr. range from <2 to 6 log CFU / g (Fasolato *et al*. 2018) and *C. perfringens* at 3.3 log CFU / g (Kurdi *et al*. 2021). Generally, there are only few studies on silkworms as edible insects, but the microbial load resembles that of other insects.

4.4 Fate of microorganisms in edible insects depending on treatment

Edible insects are known to have a naturally high microbial load when harvested, while the reported findings of the significance of substrate contaminated with pathogens and the elimination effect of post-harvest processing methods are more diverse. Besides, several studies address the dynamics of microbiota composition in insects and how it is influenced by type of feeding substrate, and the immune response to various pathogens in particularly BSFL and yellow mealworm.

It seems that BSFL undergoing bacterial challenge elicit immune responses that vary depending on species or group of bacteria and further influence the degree and speed of elimination of specific intruding bacteria. For example, Gram-positive bacteria persisted longer in the haemolymph of BSFL after injection than Gram-negative bacteria although both Gram-positive- and Gram-negative bacteria were completely removed from the insect body within a few hours after injection (Bruno *et al*. 2021). Production of various antimicrobial peptides further helps BSFL to fight off pathogens (Wu *et al.* 2018; Ho *et al*. 2021; van Moll *et al*. 2022). The gut associated microorganisms of BSFL also seem to support suppression of pathogens like *Salmonella* and *Staphylococcus* in waste substrate (Zhang *et al* 2022). However, this substrate sanitation capacity of BSFL varies for both the type of substrate and the bacteria species (Gold *et al.* 2018; Awasthi *et al.* 2020; Lopes *et al*. 2020; Swinscoe *et al*. 2020).

Moreover, the type of substrate may induce a shift in gut microbiota of BSFL (Bruno *et al*. 2019; Calassi *et al*. 2021; Schreven *et al*. 2022) and especially fungal communities were highly substrate dependent in

a Kenyan study (Tanga *et al*. 2021). A diet consisting of spent grains led for example to a predominance of the fungus *Pichia kudriazevii,* which has a safety perspective as this fungus encodes an antibacterial toxin active against human pathogens *E. coli, Enterococcus faecalis* and *Staphylococcus aureus.* However, also BSFL core microorganisms seem to prevail irrespective of the type of substrate, although this core may differ regionally (Klammsteiner *et al*. 2020; Shelomi *et al*. 2020; Osimani *et al*. 2021; Gorrens *et al*. 2022).

Regardless, it is generally agreed that the high intrinsic load of bacteria in edible insects implies a need for efficient means to reduce this load, as well as ways to stabilize insect products to prevent proliferation of undesired bacteria, to ensure the food and feed safety.

Before harvest of the insects, starving is commonly applied to empty the gut content, but this has generally minimal or no effect on the microbial load. However, Mancini *et al*. (2019c) reported fewer Enterobacteriaceae, CPS, endospores and yeast and moulds after 24 h fasting, but the effect was not consistent for all types of substrates or species.

Freezing is a common method applied to kill insects, but it does not seem to reduce the microbial load. However, insect samples are often frozen before performance of the microbiological analysis, which may affect the numbers recovered of more sensitive species and does not allow a direct comparison of the load in fresh *vs*. frozen insects.

Heat treatment of edible insects, e.g. blanching in boiling water or lower temperatures, and oven drying and/or roasting is applied as single treatment or in various combinations of temperature ranges and duration. The latter makes it difficult to deduce the elimination effect of the individual treatment steps. Furthermore, the bacterial species evaluated vary between studies as well as the level of bacteria before the heat treatment.

In some studies, blanching of edible insects 40 to 60 sec in boiling water reduces the bacterial level to about 2 log CFU / g (Borremans *et al*. 2018; Nga' ang' a *et al*. 2021), while another study still found > 6 log CFU / g total aerobic bacteria (TAC) in BSFL even after 8 min of scalding in boiling water (Saucier *et al*. 2022). The level of endospores was not determined in the later study, but due to their resistance to heat, it seems plausible that the TAC consists of such endospores. However, even bacteria like Enterobacteriaceae and *Listeria* spp. were found in levels of > 5 log CFU / g after the 8 minutes of scalding. Moreover, despite the expected heat resistance of endospores, one study reported that endospores were below the level of detection (< 1 log CFU / g) in yellow mealworm after treatment at 60°C for 5 min even though the total aerobic count was still 5.6 log CFU / g (Mancini *et al*. 2019a).

Oven drying treatments at 60°C may reduce the microbial load to some extent depending on the duration of treatment (Larouche *et al*. 2019; Nyangena *et al* 2020; Ng'ang'a *et al* 2021). For oven treatments of yellow mealworm at 150°C for 10 min, both *Listeria* and endospores were found to decline below the detection limit (< 1 log CFU / g) although TAC levels were still 3-4 log CFU /g (Mancini *et al*. 2019ac, 2022). In studies where yellow mealworm meal containing 2-2.8 log CFU / g endospores was incorporated (10% or 30%) into dough and baked for 1 h at 200°C, the level of endospores in the bread was <1 or 0,58-1.23 log CFU / g (Roncolini *et al*. 2019, 2020). In another study, where cricket meal containing 5.5 log CFU / g endospores was baked in bread for 1 h at 200°C, the bread still contained up to 3.7 log CFU / g (Osimani *et al*. 2018).

Generally, there is a lack of well-evaluated and standardized methods for the gentlest treatment concerning product quality that at the same time ensures a high level of food safety of insect products. The various heating methods differ with respect to heat transfer efficiency that further depends on the morphology of the target insect. This implies a need for specific assessments of each method and insect product to prove the efficiency of the applied treatment for lowering the microbial load and specific pathogens. In relation to the microbiological analyses applied, there is also a lack of standardized methods for homogenization of the insect sample as it may influence the number of bacteria recovered, which impairs comparisons across studies.

Irrespective of the somewhat variable results regarding the consequence of rearing insects on substrates with a poor hygienic level in terms of resulting contamination in the harvested insects, the sanitation capacity of postharvest processing steps will determine the food safety of the final insect product. Consequently, microbial contaminants not easily controlled or eliminated from the insect after harvest should be avoided or eliminated already in the substrate.

5 Chemical hazards

Chemical hazards included in this review are: metals and other elements, mycotoxins, pesticides, dioxins and PCBs, and finally other compounds of interest (minerals, oils, PFAS).

This literature review is limited to describe transfer of chemical hazards from substrate to insect larvae, and bioaccumulation of chemical hazards in larvae. The review does not cover metabolism or possible toxic effects of the chemical hazards.

The review focuses on individual studies and does not cover review papers. There are several relevant reviews (Alagappan *et al*., 2022; Meyer *et al*, 2021; Lievens *et al*., 2021; Purnamasari *et al*., 2022; Schrögel and Wätjen, 2019; van der Fels-Klerx *et al*., 2018) focusing on chemical hazards, rearing of insect, and feed and food safety, which are referred to as a supplement to this review.

5.1 Metals and other elements

Several studies have investigated the transfer and bioaccumulation of metals and other elements in insect larvae, mainly in BSFL. Focus has largely been on the heavy metals cadmium (Cd), lead (Pb) and mercury (Hg), and the metalloid arsenic (As). Other elements of interest were aluminium (Al), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn). Two studies focus on yellow mealworm. In one study, As, Cd and Pb were transferred from spiked substrates, but only arsenic bioaccumulated in yellow mealworm (van der Fels-Klerx *et al*., 2016). Truzzi and colleagues studied the transfer of As, Cd, Hg, Ni, Pb and Se from substrates containing solid residues from the processing of olive fruits. They found that the elements are transferred, but results suggest that only Hg bioaccumulated in the yellow mealworm (Truzzi *et al*., 2019). Studies on BSFL suggest that elements are transferred from substate (spiked and non-spiked) to BSFL, and that in particular Cd is bioaccumulated, however, there are differences across the studies (Biancarosa *et al.*, 2018; Bohm *et al*., 2022; Bulak *et al*., 2018; Diener *et al.*, 2015; Elechi *et al*., 2021; Gao *et al.*, 2017; Proc *et al*., 2020; Purschke *et al.*, 2017; Truzzi *et al.*, 2020; van der Fels-Klerx *et al.*, 2016; van der Fels-Klerx *et al.*, 2020; Wu *et al.*, 2020). A few of the later studies on BSFL have used non-spiked substrates focusing on the use of former foodstuffs or by-products from food production in substrates for larvae. Substrates based on by-products from coffee roasting with or without microalgae contained As, Cd, Hg, Ni and Pb; the elements were transferred to the BSFL and Cd, Hg and Pb bioaccumulated in the larvae (Truzzi *et al*. 2020). Three types of organic waste (brewery water, food waste (mainly rice, beans, plantain, and vegetables) and fruit waste) containing varying levels of Cd, Cr, Cu, Pb and Zn were fed to BSFL; the elements

were transferred from the substrates to the larvae, and Cd in food waste and fruit waste, but not in the brewery waste, bioaccumulated (Elechi *et al*., 2021). A recent study covered 12 elements and various types of substrates, including household food waste (cuts and peels of fruit and vegetables, grounded tea, rice, noodles, and eggs shells) (Bohm *et al*., 2022). The elements Cu, Mg, Mn, Pg, Zn, and in particular Cd, bioaccumulated in BSFL reared on the food waste substrate (Bohm *et al*., 2022). Fels-Klerx and colleagues (2020) produced substrates mimicking former foodstuffs with or without packaging materials; the four substrates were: meat and paperboard carton, vegetable and paperboard carton, meat and plastic, and vegetable and plastic. The levels of As and Hg were very low, often below the limits of quantification (LOQ) in the substrates and BSFL fed on the substrates (van der Fels-Klerx *et al*., 2020). The substrates contained Cd, but the levels of Pb were below the LOQ. Both Cd and Pb were found in the BSFL, and for all substrates Cd bioaccumulated (van der Fels-Klerx *et al*., 2020).

5.2 Mycotoxins

Several studies have investigated the transfer and bioaccumulation of mycotoxins in insect larvae, mainly in BSFL and yellow mealworm. The studies cover the following mycotoxins: Aflatoxin A, deoxynivalenol (DON), zearalenone, ochratoxin A, fumonisin B1 + B2, Sum T-2 and HT-2 toxin. The studies suggest that although some mycotoxins are transferred from the substates (spiked and non-spiked) to the larvae, the larvae do not bioaccumulate the mycotoxins (Bosch *et al.*, 2017; Camenzuli *et al.*, 2018; Charlton *et al.*, 2015; Gulsunoglu *et al.*, 2019; Leni *et al.*, 2019; Mancini *et al*., 2020; Meijer *et al.*, 2019; Niermans *et al.*, 2019; Piacenza *et al*., 2021; Purschke *et al.*, 2017; Sanabria *et al.*, 2019; Van Broekhoven *et al.*, 2017). Some of the studies investigated the transfer of mycotoxins from naturally contaminated substrates to insect larvae. Grains infected with *Fursarium* were fermented before being fed to BSFL (Gulsunoglu *et al*., 2019). The substrate contained DON, which was transferred to the BSFL at low level, but was not bioaccumulated by the larvae (Gulsunoglu *et al*., 2019). Leni and colleagues (2019) prepared substrates with ingredients naturally contaminated with DON, fumonisin B1, fumonisin B2 and/or zearalenone, and reared BSFL or *Alphitobius diaperinus* (lesser mealworm) on the substrates. The mycotoxins were not detected in the BSFL, while DON and fumonisin B1 were detected at low levels in lesser mealworm (Leni *et al*., 2019). The levels in the lesser mealworm were lower than in the substrates, indicating that DON and fumonisin B1 did not bioaccumulate. The transfer of DON from naturally contaminated substrates to larvae has been studied in yellow mealworm. In one study, yellow mealworm was fed a substrate based on naturally contaminated wheat flour, and although DON was present in the substrate, it was not detected in the mealworm (Van Broekhoven *et al*., 2017). However,

in a similar study, where yellow mealworm was fed substrates containing wheat contaminated with DON, DON was found in low levels in the mealworm (Sanabria *et al*., 2019). In a third study on yellow mealworm, oat flakes naturally contaminated with T-2 and HT-2 were fed to mealworm, but the two mycotoxins were not detected in the mealworm (Piacenza *et al*., 2021). Similarly, zeralenone was not found in yellow mealworm after being reared on a substrate containing naturally contaminated wheat flour (Niermans *et al*., 2019).

This literature review was limited to describing transfer of mycotoxins from substrate to insect larvae, and bioaccumulation of mycotoxins in larvae. Many of the studies mentioned above also looked at metabolism and excretion of mycotoxins by larvae, indicating that insect larvae metabolise and excrete mycotoxins. A recent review by Niermans and colleagues (2021) describes the accumulation, metabolism, and excretion of mycotoxins in insects.

5.2.1 Assessment of risks related to mycotoxins and the feeding of insects with spoiled former foodstuffs or spoiled food waste.

Former foodstuffs and food waste, spoiled and non-spoiled, may contain mycotoxins, which may cause adverse health effects in animals and humans. This assessment is limited to insects fed substrates with spoiled food waste or spiled former foodstuffs. Risks for other farmed animals and humans have been thoroughly assessed by EFSA over the years (2004 and onwards). For more information on risks to farmed animals (other than insects) and humans, readers are referred to EFSA's risk assessments of mycotoxins in feed and food (www.efsa.eu).

Former foodstuffs such as cereals, bread, fruit, and cheeses may contain mycotoxins. In food waste possible sources of mycotoxins are fruit, vegetables, cereals, bread, nuts, seeds, jam, marmalade, cheeses, etc. Other types of organic waste, that could potentially be used in insect substrates are downgraded grains/cereals, i.e., batches of grain that a downgraded from use in food production due to elevated levels of mycotoxins.

Niermans and colleagues (2021) conducted a systematic literature review of the accumulation and metabolism of mycotoxins in insects, and of the effects of mycotoxin exposure on growth and survival of insects. Some of the major findings of the systematic review were: 1. The accumulation of mycotoxins is low in most insects, 2. Mycotoxins are metabolised by insects, and 3. The effects of mycotoxins on growth and survival depend on insect species, type of mycotoxin and concentration of the mycotoxin(s), as well as life stage of the insect(s). The authors states that "Results of the review support an optimistic

outlook for the use of mycotoxin-contaminated waste streams as substrate for insect rearing" (Niermans *et al*., 2021).

Studies in the scientific literature (see 5.2) find that although some mycotoxins are transferred from substrate to insects, the accumulation is low, and the insects do not bioaccumulate the mycotoxins. The exposure to mycotoxins does not seem to affect the insects. In studies where insect larvae were fed substrates with ingredients naturally contaminated with mycotoxins, growth and survival of the insects were not affected (Gulsunoglu *et al*., 2019; Niermans *et al*., 2019; Piacenza *et al*., 2021; Sanabria *et al*., 2019; Van Broekhoven *et al*., 2017). When BSFL were fed a substate containing grains infected with DON, their weight gain and survival rate did not differ significantly from BSFL fed a control substate with non-contaminated grains (Gulsunoglu *et al*., 2017). Similar observations were seen in studies with yellow mealworm; when reared on substrates containing ingredients naturally contaminated with DON or T-2 and HT-2, growth and survival of the exposed mealworms were comparable to those reared on control substates (Piacenza *et al*., 2021; Sanabria *et al*., 2019; Van Broekhoven *et al*., 2017). In one study on yellow mealworm, the weight gain of mealworms increased when exposed zeralenone (naturally contaminated substrate) compared to control mealworms, while the survival rates were similar (Niermans *et al*., 2019).

Data on the occurrence of mycotoxins in insects, substrates for insects, former foodstuffs and food waste is scarce. There are data available in the literature, and these data were reviewed by Niermans and colleagues (2021). They found that, in general, when exposed to mycotoxins, the concentration of mycotoxins in insects is below limit of detection or limit of quantification. Even at exposure levels exceeding current European Union guidance values for mycotoxins in feed (Niermans *et al*., 2021). In some studies mycotoxins accumulate in exposed insects, but the concentrations found are in most cases below European Union maximum limits or guidance values set for insects used as feed or food (Niermans *et al*., 2021).

5.3 Pesticides

Only a few studies examine how pesticides in the substrate affect the content of pesticides in BSFL and yellow mealworm. Charlton and colleagues (2015) studied the levels of several chemical contaminants, including a screening of 393 pesticides, in four species of insects (house fly, blue bottle, blow fly and BSFL) reared on substrates of "low or zero value waste materials". Only two pesticides were detected in the samples; chlorpyrifos was detected in one sample of house fly larvae and piperonyl butoxide was detected in one sample of blue bottle larvae (Charlton *et al*., 2015). The transfer of pesticides from

spiked substrate to insects has been studied in BSFL and in yellow mealworm. Pesticides are transferred from spiked substrates to the BSFL, but there is no bioaccumulation of the pesticides (Lalander *et al*., 2016; Meijer *et al*., 2021; Purschke *et al*., 2017). In yellow mealworm, pesticides are also transferred from spiked substrates to the mealworm, and results indicate that some pesticides may accumulate in the mealworm (Houbraken *et al*., 2016; Dreassi *et al*., 2020). The studies covered the following pesticides; 1. Azoxystrobin and propiconazole (Lalander *et al*., 2016), 2. Chlorpyrifos, chlorpyrifos-methyl and pirimiphos-methyl (Purschke *et al*., 2017), 3. Chlorpyrifos, Propoxur, cypermethrin, imidacloprid, spinosad and tebufenoxide (Meijer *et al*., 2021), 4. 2,4-D, bentazone, bifenthrin, clopyralid, diflufenican, fenpropimorph, isoproturon, linuron, mefenoxam, pendimethalin, pyrimethanil, tebuconazole (Houbraken *et al*., 2016) and 5. Deltamethrin, tebuconazole and chlormequat chloride (Dreassi *et al*., 2020).

5.4 Dioxins and PCBs

Only a few studies have looked at dioxins and PCBs (Polychlorinated Biphenyls). Charlton and colleagues (2015) studied the levels of dioxins and PCBS in four species of insects (house fly, blue bottle, blow fly and BSFL) reared on substrates of "low or zero value waste materials". Dioxins and PCBs were found in all samples, but at levels below the maximum levels set in the EU feed legislation. BSFL fed on substrates mimicking former foodstuffs (former foodstuffs with or without packaging materials; the substrates were: meat and paperboard carton, vegetable and paperboard carton, meat and plastic, and vegetable and plastic) contain dioxins and PBCs, and the estimated Bioaccumulation factor for the compounds were > 1, indicating the BSFL can accumulate dioxins and PCBs (van der Fels-Klerx *et al*., 2020).

5.5 Other compounds of interest

Food waste may contain packaging residues, such as cardboard and paper, which may contain problematic substances, e.g. fluorinated substances used in the coating of food packaging materials. Only two studies on this topic were identified. In a study with BSFL reared on substrates mimicking former foodstuffs including packaging materials (plastic and cardboard/paper), the content of mineral oils was analyzed in the larvae (van der Fels-Klerx *et al*., 2020). Mineral oils were detected in the larvae, but do not appear to bioaccumulate (van der Fels-Klerx *et al*., 2020). The transfer of PFAS to insect larvae has been studied in BSFL by Li and Bischel (2022). Five compounds (PFBA, PFOA, PFBS, L-PFOS and GenX) spiked to the substrate were transferred but not bioaccumulated in the BSFL (Li and Bischel, 2022).

5.6 Other hazards

Food waste, unless sorted, will contain meat. One study has studied the possible transfer of animal DNA from substrate to insect larvae; ruminant DNA was detected in BSFL reared on substrates spiked with bovine protein (bovine haemoglobin powder), indicating that animal DNA can be transferred from substate to larvae (Belghit *et al.*, 2021).

6 Identified areas requiring further research

The following identification of knowledge gabs requiring further research is mainly based on the topics described in the literature review, but also on a general discussion of available substrates and rearing of insects for feed and food production.

The identified research needs are divided in three categories: 1) research related to the substrate, 2) research related to the transfer of relevant hazards from the substrate to the insects, and the bioaccumulation of these hazards in the insects and 3) research related to post harvest treatment of the insects.

6.1 Research needs related to substrates for rearing of insects.

- Identification of relevant by-products, food waste and former foodstuff for use as substrate for insects, based on availability in terms of accessibility and amounts over seasons as well as appropriateness as feed for the insect of interest in terms of composition and safety.
- Characterization of selected substrate candidates by:
    - screening for the content of relevant chemical compounds, including regulated compounds and emerging contaminants.
    - screening for the level of bacterial hygiene indicators and presence of pathogens (e.g. according to specified requirements for feed)
    - assessing the presence and persistence of viral indicators

      This characterization shall address the impact of seasonal variations and support provision of data for quantitative risk assessments.
- Assess the need for sanitizing treatments of substrate in order to prevent reintroduction of hazards back into the food production chain – addressing appropriate treatment methods, considering the sanitation efficiency and quality of the resulting substrate, as well as the significance of timing (i.e. at which stage should a sanitizing step be implemented).
- Assess the effect of storage conditions e.g. temperature as a means to control microbiological hazards such as toxin producing moulds.

6.2 Research needs related to the transfer of relevant hazards from the substrate to the insects.

- Experimental assessments of the possible transfer and bioaccumulation of relevant chemical compounds e.g.

    - Pesticides, PFAS, or compounds in food contact materials
    - Compounds identified in surveys of food waste (see 1 above)
- Experimental assessments of the transfer of relevant microbiological hazards, their persistency, and the effect of the contamination level in the substrate e.g.
    - Spore forming bacteria and toxin producing bacteria (knowledge on dynamics of toxin production)
    - Virus indicators (will they remain infective)

6.3 Research needs related to the processing of insects.

- Determine required postharvest treatment methods for each insect of interest for efficient reduction of the naturally high microbial load and potential pathogens if present.
- Elucidate the effect of suggested processing methods on the nutritional quality of insect product, including possible formation of process contaminants.
- Assess if the applied treatment affects the level of chemical compounds, for example mycotoxins.

7 References

Abdollahi, L., Munkholm, L.J., 2014. Tillage system and cover crop effects on soil quality: I. chemical, mechanical, and biological properties. Soil Science Society of America Journal 78, 262-270

Abdollahi, L., Munkholm, L.J., Garbout, A., 2014. Tillage system and cover crop effects on soil quality: II. pore characteristics. Soil Science Society of America Journal 78, 271-279

Alagappan, S., Rowland, D., Barwell, R., Cozzolino, D., Mikkelsen, D., Olarte Mantilla, S.M., James, P., Yarger, O., Hoffman, L., 2022. Organic side streams (bioproducts) as substrate for black soldier fly (*Hermetia illucens*) intended as animal feed: Chemical safety issues. Animal Production Science. https://doi.org/10.1071/AN22155

Anonymous, 2022. Annual Report on Zoonoses in Denmark 2021, National Food Institute, Technical University of Denmark

Awasthi, M.K., Liu, T., Awasthi, S.K., Duan, Y., Pandey, A., Zhang, Z., 2020. Manure pretreatments with black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae): A study to reduce pathogen content. Science of the Total Environment 737. https://doi.org/10.1016/j.scitotenv.2020.139842

Bawa, M., Songsermpong, S., Kaewtapee, C., Chanput, W., 2020. Effects of microwave and hot air oven drying on the nutritional, microbiological load, and color parameters of the house crickets (*Acheta domesticus*). Journal of Food Processing and Preservation 44. https://doi.org/10.1111/jfpp.14407

Belghit, I., Varunjikar, M., Lecrenier, M.-C., Steinhilber, A.E., Niedzwiecka, A., Wang, Y.V., Dieu, M., Azzollini, D., Lie, K., Lock, E.-J., Berntssen, M.H.G., Renard, P., Zagon, J., Fumière, O., van Loon, J.J.A., Larsen, T., Poetz, O., Braeuning, A., Palmblad, M., Rasinger, J.D., 2021. Future feed control – Tracing banned bovine material in insect meal. Food Control 108183. https://doi.org/10.1016/j.foodcont.2021.108183

Belleggia, L., Milanović, V., Cardinali, F., Garofalo, C., Pasquini, M., Tavoletti, S., Riolo, P., Ruschioni, S., Isidoro, N., Clementi, F., Aquilanti, L., Osimani, A., 2020. Listeria dynamics in a laboratory-scale food chain of mealworm larvae (*Tenebrio molitor*) intended for human consumption. Food Control 114. https://doi.org/10.1016/j.foodcont.2020.107246

Bessa, L.W., Pieterse, E., Marais, J., Dhanani, K., Hoffman, L.C., 2021. Food safety of consuming black soldier fly (*Hermetia illucens*) larvae: Microbial, heavy metal and cross-reactive allergen risks. Foods 10. https://doi.org/10.3390/foods10081934

Biancarosa, I., Liland, N.S., Biemans, D., Araujo, P., Bruckner, C.G., Waagbø, R., Torstensen, B.E., Lock, E.J., Amlund, H., 2018. Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media. Journal of the Science of Food and Agriculture 98, 2176–2183. https://doi.org/10.1002/jsfa.8702

Bohm, K., Hatley, G.A., Robinson, B.H., Gutiérrez-Ginés, M.J., 2022. Black Soldier Fly-based bioconversion of biosolids creates high-value products with low heavy metal concentrations. Resources, Conservation and Recycling 180, 106149. https://doi.org/10.1016/j.resconrec.2021.106149

Bonnet, S.I., Bouhsira, E., De Regge, N., Fite, J., Etoré, F., Garigliany, M.-M., Jori, F., Lempereur, L., Le Potier, M.-F., Quillery, E., Vergne, T., Vial, L., 2020. Putative Role of arthropod vectors in African swine fever virus transmission in relation to their bio-ecological properties. Viruses 12. https://doi.org/10.3390/v12070778

Borremans, A., Lenaerts, S., Crauwels, S., Lievens, B., Van Campenhout, L., 2018. Marination and fermentation of yellow mealworm larvae (*Tenebrio molitor*). Food Control 92, 47–52. https://doi.org/10.1016/j.foodcont.2018.04.036

Bosch, G., Fels-Klerx, H., Rijk, T., Oonincx, D., 2017. Aflatoxin B1 Tolerance and Accumulation in Black Soldier Fly Larvae (*Hermetia illucens*) and Yellow Mealworms (*Tenebrio molitor*). Toxins 9, 185. https://doi.org/10.3390/toxins9060185

Bruno, D., Montali, A., Mastore, M., Brivio, M.F., Mohamed, A., Tian, L., Grimaldi, A., Casartelli, M., Tettamanti, G., 2021. Insights Into the Immune Response of the Black Soldier Fly Larvae to Bacteria. Frontiers in Immunology 12. https://doi.org/10.3389/fimmu.2021.745160

Bulak, P., Polakowski, C., Nowak, K., Waśko, A., Wiącek, D., Bieganowski, A., 2018. *Hermetia illucens* as a new and promising species for use in entomoremediation. Science of the Total Environment 633, 912–919. https://doi.org/10.1016/j.scitotenv.2018.03.252

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 B1, zearalenone, deoxynivalenol, and ochratoxin A by *Alphitobius diaperinus* and *Hermetia illucens* from contaminated substrates. Toxins 10, 91. https://doi.org/10.3390/toxins10020091

Cappelli, A., Cini, E., Lorini, C., Oliva, N., Bonaccorsi, G., 2020. Insects as food: A review on risks assessments of Tenebrionidae and Gryllidae in relation to a first machines and plants development. Food Control 108, 106877. https://doi.org/10.1016/J.FOODCONT.2019.106877

Charlton, A.J., Dickinson, M., Wakefield, M.E., Fitches, E., Kenis, M., Han, R., Zhu, F., Kone, N., Grant, M., Devic, E., Bruggeman, G., Prior, R., Smith, R., 2015. Exploring the chemical safety of fly larvae as a source of protein for animal feed. Journal of Insects as Food and Feed 1, 7–16. https://doi.org/10.3920/JIFF2014.0020

De Smet, J., Vandeweyer, D., Van Moll, L., Lachi, D., Van Campenhout, L., 2021. Dynamics of *Salmonella* inoculated during rearing of black soldier fly larvae (*Hermetia illucens*). Food Research International 149. https://doi.org/10.1016/j.foodres.2021.110692

Dicke, M., Eilenberg, J., Salles, J.F., Jensen, A.B., Lecocq, A., Pijlman, G.P., van Loon, J.J.A., van Oers, M.M., 2020. Edible insects unlikely to contribute to transmission of coronavirus SARS-CoV-2. Journal of Insects as Food and Feed 6, 333–339. https://doi.org/10.3920/JIFF2020.0039

Diener, S., Zurbrügg, C., Tockner, K., 2015. Bioaccumulation of heavy metals in the black soldier fly, *Hermetia illucens* and effects on its life cycle. Journal of Insects as Food and Feed 1, 261–270. https://doi.org/10.3920/JIFF2015.0030

Dreassi, E., Mancini, A., Corbini, G., Botta, M., Mancini, A., Botta, M., Tarchi, F., Francardi, V., 2020. Bioaccumulation of deltamethrin, tebuconazole and chlormequat chloride in T. molitor larvae and risks associated with their human consumption. Journal of Insects as Food and Feed 6, 457–465. https://doi.org/10.3920/JIFF2020.0007

DVFA 2019. Rapport om kontrol i 2018 for salmonella og campylobacter i danskproduceret og importeret fersk kød - case-by-case kontrollen. Danish Veterinary and Food Administration.

Elechi, M.C., Kemabonta, K.A., Ogbogu, S.S., Orabueze, I.C., Adetoro, F.A., Adebayo, H.A., Obe, T.M., 2021. Heavy metal bioaccumulation in prepupae of black soldier fly Hermetia illucens (Diptera: Stratiomyidae) cultured with organic wastes and chicken feed. International Journal of Tropical Insect Science 41, 2125–2131. https://doi.org/10.1007/S42690-021-00427-5

EFSA 2018. EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fern_andez Esc_amez PS, Giron_es R, Herman L, Koutsoumanis K, Lindqvist R, Nørrung B, Robertson L, Ru G, Sanaa M, Skandamis P, Snary E, Speybroeck N, Kuile BT, Threlfall J, Wahlstr€om H, Adkin A, Greiner M, Marchis D, Prado M, Da Silva Felicio T, Ortiz-Pelaez A and Simmons M, 2018. Scientific opinion on an updated quantitative risk assessment (QRA) of the BSE risk posed by processed animal protein (PAP). EFSA Journal 2018 16, e5314. https://doi.org/10.2903/j.efsa.2018.5314

Fasolato, L., Cardazzo, B., Carraro, L., Fontana, F., Novelli, E., Balzan, S., 2018. Edible processed insects from e-commerce: Food safety with a focus on the Bacillus cereus group. Food Microbiology 76, 296–303. https://doi.org/10.1016/j.fm.2018.06.008

Fischer, M., Mohnke, M., Probst, C., Pikalo, J., Conraths, F.J., Beer, M., Blome, S., 2020. Stability of African swine fever virus on heat-treated field crops. Transboundary and Emerging Diseases 67, 2318–2323. https://doi.org/10.1111/tbed.13650

Frentzel, H., Kelner-Burgos, Y., Fischer, J., Heise, J., Göhler, A., Wichmann-Schauer, H., 2022. Occurrence of selected bacterial pathogens in insect-based food products and in-depth characterisation of detected *Bacillus cereus* group isolates. International Journal of Food Microbiology 379. https://doi.org/10.1016/j.ijfoodmicro.2022.109860

Fröhling, A., Bußler, S., Durek, J., Schlüter, O.K., 2020. Thermal Impact on the Culturable Microbial Diversity Along the Processing Chain of Flour From Crickets (*Acheta domesticus*). Frontiers in Microbiology 11. https://doi.org/10.3389/fmicb.2020.00884

Galassi, G., Jucker, C., Parma, P., Lupi, D., Crovetto, G.M., Savoldelli, S., Colombini, S., 2021. Impact of Agro-industrial Byproducts on Bioconversion, Chemical Composition, in vitro Digestibility, and Microbiota of the Black Soldier Fly (Diptera: Stratiomyidae) Larvae. Journal of Insect Science 21. https://doi.org/10.1093/jisesa/ieaa148

Gao, Q., Wang, X., Wang, W., Lei, C., Zhu, F., 2017. Influences of chromium and cadmium on the development of black soldier fly larvae. Environmental Science and Pollution Research 24, 8637–8644. https://doi.org/10.1007/S11356-017-8550-3/FIGURES/4

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 Research International 125. https://doi.org/10.1016/j.foodres.2019.108527

Gold, M., Tomberlin, J.K., Diener, S., Zurbrügg, C., Mathys, A., 2018. Decomposition of biowaste macronutrients, microbes, and chemicals in black soldier fly larval treatment: A review. Waste Management 82, 302–318. https://doi.org/10.1016/j.wasman.2018.10.022

Gorrens, E., van Looveren, N., van Moll, L., Vandeweyer, D., Lachi, D., de Smet, J., van Campenhout, L., 2021. *Staphylococcus aureus* in substrates for black soldier fly larvae (*Hermetia illucens*) and its dynamics during rearing. Microbiology Spectrum 9. https://doi.org/10.1128/SPECTRUM.02183-21

Grisendi, A., Defilippo, F., Lucchetti, C., Listorti, V., Ottoboni, M., Dottori, M., Serraino, A., Pinotti, L., Bonilauri, P., 2022. Fate of *Salmonella enterica* Typhimurium and *Listeria monocytogenes* in Black Soldier Fly (*Hermetia illucens*) Larvae Reared on Two Artificial Diets. Foods 11. https://doi.org/10.3390/foods11152208

Gulsunoglu, Z., Aravind, S., Bai, Y., Wang, L., Kutcher, H.R., Tanaka, T., 2019. Deoxynivalenol (DON) accumulation and nutrient recovery in black soldier fly larvae (*Hermetia illucens*) fed wheat infected with *Fusarium* spp. Fermentation 5. https://doi.org/10.3390/FERMENTATION5030083

Ho, P.N., Klanrit, P., Hanboonsong, Y., Yordpratum, U., Suksawat, M., Kulthawatsiri, T., Jirahiranpat, A., Deewai, S., Mackawan, P., Sermswan, R.W., Wangwiwatsin, A., Phetcharaburanin, J., 2021. Bacterial challenge-associated metabolic phenotypes in *Hermetia illucens* defining nutritional and functional benefits. Scientific Reports 11. https://doi.org/10.1038/s41598-021-02752-8

Houbraken, M., Spranghers, T., De Clercq, P., Cooreman-Algoed, M., Couchement, T., De Clercq, G., Verbeke, S., Spanoghe, P., 2016. Pesticide contamination of *Tenebrio molitor* (Coleoptera: Tenebrionidae) for human consumption. Food Chemistry 201, 264–269. https://doi.org/10.1016/j.foodchem.2016.01.097

Jensen, A.N., Hansen, S.H., Baggesen, D.L., 2020. *Salmonella* Typhimurium Level in Mealworms (*Tenebrio molitor*) After exposure to contaminated substrate. Frontiers in Microbiology 11. https://doi.org/10.3389/fmicb.2020.01613

Jensen, K., Berggreen, I.E., Amlund, H. & Nørgaad, J.V., 2022. Feeding experiments with insects and assessment of feed-related risks from kitchen- and food waste and possibly other by-products. Advisory report from DCA – National Center for Food and Agriculture, Aarhus University, delivered March 2022

Jucker, C., Belluco, S., Oddon, S.B., Ricci, A., Bonizzi, L., Lupi, D., Savoldelli, S., Biasato, I., Caimi, C., Mascaretti, A., Mascaretti, A., Gasco, L., 2022. Impact of some local organic by-products on *Acheta domesticus* growth and meal production. Journal of Insects as Food and Feed 8, 631–640. https://doi.org/10.3920/JIFF2021.0121

Kashiri, M., Marin, C., Garzón, R., Rosell, C.M., Rodrigo, D., Martínez, A., 2018. Use of high hydrostatic pressure to inactivate natural contaminating microorganisms and inoculated *E. coli* O157:H7 on *Hermetia illucens* larvae. PLoS ONE 13. https://doi.org/10.1371/journal.pone.0194477

Klammsteiner, T., Walter, A., Bogataj, T., Heussler, C.D., Stres, B., Steiner, F.M., Schlick-Steiner, B.C., Arthofer, W., Insam, H., 2020. The core gut microbiome of black soldier fly (*Hermetia illucens*) larvae raised on low-bioburden diets. Frontiers in Microbiology 11. https://doi.org/10.3389/fmicb.2020.00993

Kurdi, P., Chaowiwat, P., Weston, J., Hansawasdi, C., 2021. Studies on microbial quality, protein yield, and antioxidant properties of some frozen edible insects. International Journal of Food Science 2021. https://doi.org/10.1155/2021/5580976

Lalander, C., Senecal, J., Gros Calvo, M., Ahrens, L., Josefsson, S., Wiberg, K., Vinnerås, B., 2016. Fate of pharmaceuticals and pesticides in fly larvae composting. Science of the Total Environment 565, 279–286. https://doi.org/10.1016/j.scitotenv.2016.04.147

Larouche, J., Deschamps, M.-H., Saucier, L., Lebeuf, Y., Doyen, A., Vandenberg, G.W., 2019. Effects of killing methods on lipid oxidation, colour and microbial load of black soldier fly (*Hermetia illucens*) larvae. Animals 9. https://doi.org/10.3390/ani9040182

Leni, G., Cirlini, M., Jacobs, J., Depraetere, S., Gianotten, N., Sforza, S., Dall'Asta, C., 2019. Impact of Naturally Contaminated Substrates on Alphitobius diaperinus and *Hermetia illucens*: Uptake and excretion of mycotoxins. Toxins 11, 476. https://doi.org/10.3390/toxins11080476

Li, T.H., Zhang, C.R., Che, P.F., Ma, Y., Zang, L.S., 2021. Recycling of spent mushroom substrate and food waste: Utilisation as feed materials for black soldier Fly (*Hermetia illucens* (L.) Diptera: Stratiomyidae). Journal of Insects as Food and Feed 7, 409–417. https://doi.org/10.3920/JIFF2020.0105

Li, W., Bischel, H.N., 2022. Are resource recovery insects safe for feed and food? A screening approach for bioaccumulative trace organic contaminants. Science of the Total Environment 837. https://doi.org/10.1016/j.scitotenv.2022.155850

Lievens, S., Poma, G., De Smet, J., Van Campenhout, L., Covaci, A., Van Der Borght, M., 2021. Chemical Safety of Black Soldier Fly Larvae (*Hermetia illucens*), Knowledge Gaps and Recommendations for Future Research: a Critical Review. Journal of Insects as Food and Feed 7, 383–396. https://doi.org/10.3920/JIFF2020.0081

Lopes, I.G., Lalander, C., Vidotti, R.M., Vinnerås, B., 2020. Reduction of Bacteria in Relation to Feeding Regimes When Treating Aquaculture Waste in Fly Larvae Composting. Frontiers in Microbiology 11. https://doi.org/10.3389/fmicb.2020.01616

Machado, R.A.M., Perry, J.J., 2020. Variability in the microbial profile of retail cricket powders in the U.S. retail market. Food Protection Trends 40, 407–412.

Mancini, A., Dreassi, E., Botta, M., Tarchi, F., Francardi, V., 2020. Bioaccumulation risk assessment of aflatoxin B1, ochratoxin and fumonisin b1 in *Tenebrio molitor* larvae. Redia 103, 101–108. https://doi.org/10.19263/REDIA-103.20.16

Mancini, S., Fratini, F., Tuccinardi, T., Turchi, B., Nuvoloni, R., Paci, G., 2019a. Effects of different blanching treatments on microbiological profile and quality of the mealworm (*Tenebrio molitor*). Journal of Insects as Food and Feed 5, 225–234. https://doi.org/10.3920/JIFF2018.0034

Mancini, S., Fratini, F., Turchi, B., Mattioli, S., Dal Bosco, A., Tuccinardi, T., Nozic, S., Paci, G., 2019c. Former foodstuff products in *Tenebrio molitor* rearing: Effects on growth, chemical composition, microbiological load, and antioxidant status. Animals 9. https://doi.org/10.3390/ani9080484

Mancini, S., Paci, G., Ciardelli, V., Turchi, B., Pedonese, F., Fratini, F., 2019b. Listeria monocytogenes contamination of *Tenebrio molitor* larvae rearing substrate: Preliminary evaluations. Food Microbiology 83, 104–108. https://doi.org/10.1016/j.fm.2019.05.006

Meijer, N., de Rijk, T., van Loon, J.J.A., Zoet, L., van der Fels-Klerx, H.J., 2021. Effects of insecticides on mortality, growth and bioaccumulation in black soldier fly (*Hermetia illucens*) larvae. PloS one 16, e0249362. https://doi.org/10.1371/journal.pone.0249362

Meijer, N., Stoopen, G., Van Der Fels-Klerx, H.J., Van Loon, J.J.A., Carney, J., Bosch, G., 2019. Aflatoxin B1 Conversion by Black Soldier Fly (*Hermetia illucens*) Larval Enzyme Extracts. Toxins 11. https://doi.org/10.3390/TOXINS11090532

Meyer, A.M., Meijer, N., Hoek-van den Hil, E.F., van der Fels-Klerx, H.J., 2021. Chemical food safety hazards of insects reared for food and feed. Journal of Insects as Food and Feed 1–10. https://doi.org/10.3920/JIFF2020.0085

Mirza Alizadeh, A., Jazaeri, S., Shemshadi, B., Hashempour-Baltork, F., Sarlak, Z., Pilevar, Z., Hosseini, H., 2018. A review on inactivation methods of Toxoplasma gondii in foods. Pathogens and Global Health 112, 306–319. https://doi.org/10.1080/20477724.2018.1514137

Müller, A., Wiedmer, S., Kurth, M., 2019. Risk evaluation of passive transmission of animal parasites by feeding of black soldier fly (*Hermetia illucens*) larvae and prepupae. Journal of Food Protection 82, 948–954. https://doi.org/10.4315/0362-028X.JFP-18-484

Ng'ang'a, J., Imathiu, S., Fombong, F., Vanden Broeck, J., Kinyuru, J., 2021. Effect of Dietary Supplementation with Powder Derived from Moringa Oleifera and Azadirachta Indica Leaves on Growth and Microbial Load of Edible Crickets. Journal of Insects as Food and Feed 7, 419–431. https://doi.org/10.3920/JIFF2020.0056

Niedzwiecka, A., Boucharef, L., Hahn, S., Zarske, M., Steinhilber, A., Poetz, O., Zagon, J., Seidler, T., Braeuning, A., Lampen, A., 2019. A novel antibody-based enrichment and mass spectrometry approach for the detection of species-specific blood peptides in feed matrices. Food Control 98, 141–149. https://doi.org/10.1016/j.foodcont.2018.11.036

Niermans, K., Meyer, A.M., den Hil, E.F.H. van, van Loon, J.J.A., van der Fels-Klerx, H.J., 2021. A systematic literature review on the effects of mycotoxin exposure on insects and on mycotoxin accumulation and biotransformation. Mycotoxin Research 37, 279–295. https://doi.org/10.1007/S12550-021-00441-Z

Niermans, K., Woyzichovski, J., Kröncke, 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. https://doi.org/10.1007/s12550-019-00346-y

Nordentoft, S., Fischer, C., Bjerrum, L., Heckmann, L.H., Hald, B., 2017. Reduction of *Escherichia coli, Salmonella* Enteritidis and *Campylobacter jejuni* in poultry manure by rearing of Musca domestica fly larvae. Journal of Insects as Food and Feed 3, 145–153. https://doi.org/10.3920/JIFF2016.0058

Nyangena, D.N., Mutungi, C., Imathiu, S., Kinyuru, J., Affognon, H., Ekesi, S., Nakimbugwe, D., Fiaboe, K.K.M., 2020. Effects of traditional processing techniques on the nutritional and microbiological quality of four edible insect species used for food and feed in East Africa. Foods 9. https://doi.org/10.3390/foods9050574

Olesen, A.S., Belsham, G.J., Bruun Rasmussen, T., Lohse, L., Bødker, R., Halasa, T., Boklund, A., Bøtner, A., 2020. Potential routes for indirect transmission of African swine fever virus into domestic pig herds. Transboundary and Emerging Diseases 67, 1472–1484. https://doi.org/10.1111/tbed.13538

Osimani, A., Aquilanti, L., 2021. Spore-forming bacteria in insect-based foods. Current Opinion in Food Science 37, 112–117. https://doi.org/10.1016/j.cofs.2020.10.011

Osimani, A., Milanović, V., Cardinali, F., Garofalo, C., Clementi, F., Pasquini, M., Riolo, P., Ruschioni, S., Isidoro, N., Loreto, N., Tonucci, F., Aquilanti, L., 2018. The bacterial biota of laboratory-reared edible mealworms (*Tenebrio molitor* L.): From feed to frass. International Journal of Food Microbiology 272, 49–60. https://doi.org/10.1016/j.ijfoodmicro.2018.03.001

Percipalle, M., Salvaggio, A., Pitari, G.M., Giunta, R.P., Aparo, A., Alfonzetti, T., Marino, A.M.F., 2021. Edible insects and *Toxoplasma gondii*: Is it something we need to be concerned about? Journal of Food Protection 84, 437–441. https://doi.org/10.4315/JFP-20-239

Piacenza, N., Kaltner, F., Maul, R., Gareis, M., Schwaiger, K., Gottschalk, C., 2021. Distribution of T-2 toxin and HT-2 toxin during experimental feeding of yellow mealworm (*Tenebrio molitor*). Mycotoxin Research 37, 11–21. https://doi.org/10.1007/S12550-020-00411-X

Proc, K., Bulak, P., Wiącek, D., Bieganowski, A., 2020. *Hermetia illucens* exhibits bioaccumulative potential for 15 different elements – Implications for feed and food production. Science of the Total Environment 723. https://doi.org/10.1016/j.scitotenv.2020.138125

Purnamasari, L., Khasanah, H., 2022. Black Soldier Fly (*Hermetia illucens*) as a potential agent of organic waste bioconversion. ASEAN Journal on Science and Technology for Development 39, 69–83. https://doi.org/10.29037/AJSTD.780

Purschke, B., Scheibelberger, R., Axmann, S., Adler, A., Jäger, H., 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment 34, 1410–1420. https://doi.org/10.1080/19440049.2017.1299946

Raimondi, S., Spampinato, G., Macavei, L.I., Lugli, L., Candeliere, F., Rossi, M., Maistrello, L., Amaretti, A., 2020. Effect of rearing temperature on growth and microbiota composition of *Hermetia illucens*. Microorganisms 8, 1–13. https://doi.org/10.3390/microorganisms8060902

Roncolini, A., Milanović, V., Aquilanti, L., Cardinali, F., Garofalo, C., Sabbatini, R., Clementi, F., Belleggia, L., Pasquini, M., Mozzon, M., Niro, S., Osimani, A., 2020. Lesser mealworm (*Alphitobius diaperinus*) powder as a novel baking ingredient for manufacturing high-protein, mineral-dense snacks. Food Research International 131. https://doi.org/10.1016/j.foodres.2020.109031

Roncolini, A., Milanović, V., Cardinali, F., Osimani, A., Garofalo, C., Sabbatini, R., Clementi, F., Pasquini, M., Mozzon, M., Foligni, R., Van Campenhout, L., Aquilanti, L., 2019. Protein fortification with mealworm (*Tenebrio molitor* L.) powder: Effect on textural, microbiological, nutritional and sensory features of bread. PLoS ONE 14. https://doi.org/10.1371/journal.pone.0211747

Sanabria, C.O., 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 11. https://doi.org/10.3390/toxins11050282

Saucier, L., M'ballou, C., Ratti, C., Deschamps, M.-H., Lebeuf, Y., Vandenberg, G.W., 2020. Comparison of black soldier fly larvae pre-treatments and drying techniques on the microbial load and physicochemical characteristics. Journal of Insects as Food and Feed 8, 45–64. https://doi.org/10.3920/JIFF2021.0002

Sauter-Louis, C., Conraths, F.J., Probst, C., Blohm, U., Schulz, K., Sehl, J., Fischer, M., Forth, J.H., Zani, L., Depner, K., Beer, M., Blome, S., 2021. African swine fever in wild boar in Europe—a review. Viruses 13. https://doi.org/10.3390/v13091717

Schreven, S.J.J., de Vries, H., Hermes, G.D.A., Zeni, G., Smidt, H., Dicke, M., van Loon, J.J.A., 2022. black soldier fly larvae influence internal and substrate bacterial community composition depending on substrate type and larval density. Applied and Environmental Microbiology 88. https://doi.org/10.1128/aem.00084-22

Schrögel, P., Wätjen, W., 2019. Insects for food and feed-safety aspects related to mycotoxins and metals. Foods 8, 288. https://doi.org/10.3390/foods8080288

Shelomi, M., Wu, M.-K., Chen, S.-M., Huang, J.-J., Burke, C.G., 2021. Microbes associated with black soldier fly (Diptera: Stratiomiidae) degradation of food waste. Environmental Entomology 49, 405–411. https://doi.org/10.1093/EE/NVZ164

Skovgård, H., Kristensen, K., Hald, B., 2011. Retention of *Campylobacter* (Campylobacterales: Campylobacteraceae) in the house fly (Diptera: Muscidae). Journal of Medical Entomology 48, 1202–1209. https://doi.org/10.1603/ME11061

Swinscoe, I., Oliver, D.M., Ørnsrud, R., Quilliam, R.S., 2020. The microbial safety of seaweed as a feed component for black soldier fly (*Hermetia illucens*) larvae. Food Microbiology 91. https://doi.org/10.1016/j.fm.2020.103535

Tanga, C.M., Waweru, J.W., Tola, Y.H., Onyoni, A.A., Khamis, F.M., Ekesi, S., Paredes, J.C., 2021. organic waste substrates induce important shifts in gut microbiota of black soldier fly (*Hermetia illucens* L.): Coexistence of conserved, variable, and potential pathogenic microbes. Frontiers in Microbiology 12. https://doi.org/10.3389/fmicb.2021.635881

Truzzi, C., Annibaldi, A., Girolametti, F., Giovannini, L., Riolo, P., Ruschioni, S., Olivotto, I., Illuminati, S., 2020. A chemically safe way to produce insect biomass for possible application in feed and food production. International Journal of Environmental Research and Public Health 2020, Vol. 17, Page 2121 17, 2121. https://doi.org/10.3390/IJERPH17062121

Truzzi, C., Illuminati, S., Girolametti, F., Antonucci, M., Scarponi, G., Ruschioni, S., Riolo, P., Annibaldi, A., 2019. Influence of feeding substrates on the presence of toxic metals (Cd, pb, ni, as, hg) in larvae of *Tenebrio molitor*: Risk assessment for human consumption. International Journal of Environmental Research and Public Health 16. https://doi.org/10.3390/ijerph16234815

Van Broekhoven, S., Mota 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. https://doi.org/10.3920/WMJ2016.2102

van der Fels-Klerx, H.J., Camenzuli, L., Belluco, S., Meijer, N., Ricci, A., 2018. food safety issues related to uses of insects for feeds and foods. Comprehensive Reviews in Food Science and Food Safety 17, 1172–1183. https://doi.org/10.1111/1541-4337.12385

Van Der Fels-Klerx, H.J., Camenzuli, L., Van Der Lee, M.K., Oonincx, D.G.A.B., 2016. Uptake of cadmium, lead and arsenic by *Tenebrio molitor* and *Hermetia illucens* from contaminated substrates. PLoS ONE 11, e0166186. https://doi.org/10.1371/journal.pone.0166186

van der Fels-Klerx, H.J., Meijer, N., Nijkamp, M.M., Schmitt, E., van Loon, J.J.A., 2020. Chemical food safety of using former foodstuffs for rearing black soldier fly larvae (*Hermetia illucens*) for feed and food use. Journal of Insects as Food and Feed 6, 475–488. https://doi.org/10.3920/JIFF2020.0024

van Moll, L., de Smet, J., Paas, A., Tegtmeier, D., Vilcinskas, A., Cos, P., van Campenhout, L., 2022. In vitro evaluation of antimicrobial peptides from the black soldier fly (*Hermetia illucens*) against a selection of human pathogens. Microbiology Spectrum 10. https://doi.org/10.1128/spectrum.01664-21

Vandeweyer, D., Smet, J.D., Looveren, N.V., Campenhout, L.V., 2021. Biological contaminants in insects as food and feed. Journal of Insects as Food and Feed 7, 807–822. https://doi.org/10.3920/JIFF2020.0060

Wu, N., Wang, X., Xu, X., Cai, R., Xie, S., 2020. Effects of heavy metals on the bioaccumulation, excretion and gut microbiome of black soldier fly larvae (*Hermetia illucens*). Ecotoxicology and Environmental Safety 192, 110323. https://doi.org/10.1016/J.ECOENV.2020.110323

Wu, Q., Patočka, J., Kuča, K., 2018. Insect antimicrobial peptides, a mini review. Toxins 10. https://doi.org/10.3390/toxins10110461

Wynants, E., Frooninckx, L., Crauwels, S., Verreth, C., De Smet, J., Sandrock, C., Wohlfahrt, J., Van Schelt, J., Depraetere, S., Lievens, B., Claes, J., Van Campenhout, L., 2019b. assessing the microbiota of

black soldier fly larvae (*Hermetia illucens*) reared on organic waste streams on four different locations at laboratory and large scale. Microbial Ecology 77, 913–930. https://doi.org/10.1007/s00248-018-1286-x

Wynants, E., Frooninckx, L., Van Miert, S., Geeraerd, A., Claes, J., Van Campenhout, L., 2019a. Risks related to the presence of *Salmonella* sp. during rearing of mealworms (*Tenebrio molitor*) for food or feed: Survival in the substrate and transmission to the larvae. Food Control 100, 227–234. https://doi.org/10.1016/j.foodcont.2019.01.026

Zhang, Y., Xiao, X., Elhag, O., Cai, M., Zheng, L., Huang, F., Jordan, H.R., Tomberlin, J.K., Sze, S.-H., Yu, Z., Yu, Z., Zhang, J., 2022. *Hermetia illucens* L. larvae–associated intestinal microbes reduce the transmission risk of zoonotic pathogens in pig manure. Microbial Biotechnology 15, 2631–2644. https://doi.org/10.1111/1751-7915.14113