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Low transfer of cadmium, lead and aflatoxin B1 to eggs and meat of laying hens receiving diets with black soldier fly larvae reared on contaminated substrates

M. Heuel [a], M. Kreuzer [a], I.D.M. Gangnat [a], E. Frossard [a], C. Zurbrügg [b], J. Egger [b], B. Dortmans [b], M. Gold [b,c], A. Mathys [c], J. Jaster-Keller [d], S. Weigel [d], C. Sandrock [e], M. Terranova [f,*]

[a] ETH Zurich, Institute of Agricultural Sciences, Eschikon 27, 8315 Lindau, Switzerland
[b] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Department Sanitation, Water and Solid Waste for Development (Sandec), Überlandstr. 133, 8600 Dübendorf, Switzerland
[c] ETH Zurich, Laboratory of Sustainable Food Processing, Schmelzbergstr. 9, 8092 Zurich, Switzerland
[d] German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany
[e] Research Institute of Organic Agriculture (FiBL), Departement of Livestock Science, Ackerstr. 113, 5070 Frick, Switzerland
[f] ETH Zurich, AgroVet Strickhof, Eschikon 27, 8315 Lindau, Switzerland

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ABSTRACT

Replacing soybeans with insects in egg and poultry meat production could improve environmental sustainability. Black soldier fly larvae (BSFL) have a favorable nutrient composition and can be reared on low-grade waste, but this is associated with the risk of feed and food contamination. The aim of this study was to assess the transfer of selected contaminants from larval substrates to poultry-derived food. Two different control substrates were used. Substrate C_{CH} (produced in Switzerland) was based on side streams approved for insect rearing in the European Union (EU), while substrate C_{IND} (produced in Indonesia) included non-EU approved waste. In addition, substrate C_{IND} was spiked with either heavy metals (HM; 1.9 mg cadmium and 18.8 mg lead/kg dry matter (DM)) or 1.5 mg aflatoxin B1/kg DM (AF)). The larvae fed HM contained 7 mg cadmium and 16 mg lead/kg DM. These values were about 30 times the concentrations of cadmium and 30–60 times the concentrations of lead found on average in the BSFL reared with the two non-spiked substrates. Although substrate AF contained 842 μg aflatoxin B1/kg DM as analysed, the AF larvae contained only 4 μg aflatoxin B1/kg DM. Larval meals were integrated at 200 g/kg in two control diets (diets C_{CH} and C_{IND}) and two diets based on contaminated BSFL (diets HM and AF) designed for late-laying hens (n = 9/treatment). After feeding these diets for 4 weeks, the hens were slaughtered. Diet HM and AF did not affect laying performance or egg quality compared with the control diets. In the body tissue, the cadmium concentrations (per kg DM) were nearly doubled by diet HM in the breast meat (13.3 μg), kidneys (12.3 mg) and liver (1.86 mg) compared to diet C_{IND}. The same diet increased lead in kidneys from below 0.1 to 0.5 mg/kg DM. No lead was detected in the meat and eggs, and no cadmium was found in the eggs. In

Abbreviations: AF, larvae substrate spiked with aflatoxin B1; AFB1, aflatoxin B1; AFM1, aflatoxin M1; BSFL, black soldier fly larvae; C_{CH}, control larvae substrate produced in Switzerland; Cd, cadmium; C_{IND}, control larvae substrate produced in Indonesia; DM, dry matter; EC, European Commission; EU, European Union; HM, larvae substrate spiked with heavy metals; Pb, lead.

* Corresponding author.
E-mail address: melissa-terranova@ethz.ch (M. Terranova).

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conclusion, despite cadmium and lead also occurring in BSFL meals of C_{CH} and C_{IND}, the levels in all corresponding hen-based feed and food materials were below the maximum content, except for the kidneys. The aflatoxin B1 level of diet AF (1 µg/kg DM) suggests that the risk might also be small when BSFL are reared on moldy substrate containing aflatoxin-producing fungi. In conclusion, postconsumer waste apparently poses a lower risk than expected in poultry food chains for these contaminants when used as larval substrate.

1. Introduction

The supply of feed protein that is used to produce food of animal origin currently heavily relies on soybean protein. Because of the detrimental environmental impacts of soy production, alternative protein feeds are needed. In this context, insect-based proteins are a promising alternative (van Huis, 2020) and have recently been approved for poultry feed in the EU (European Commission (EC) Regulation 2021/1372; EC, 2021). In particular, the black soldier fly larvae (BSFL, *Hermetia illucens* L.) showed a comparable or better feeding value than that of soybean-based laying hen feeds (Mwaniki et al., 2020; Heuel et al., 2021a; b, 2022). The sustainability of feed insect production is particularly high when organic wastes are used as the main rearing substrate (Smetana et al., 2019). However, the use of waste might carry the risk of introducing contaminants into the feed-food chain; therefore, rearing substrates allowed in the EU only include cereals, former food products, preconsumer products, and food production side streams (Pinotti and Ottoboni, 2021). However, the use of such substrates for insect production could be inefficient compared to their direct consumption or feeding to livestock. Outside of the EU, such as in African countries, manure and municipal waste are also frequently used for feed insect production (Münke-Svendsen et al., 2017). It has been shown that no critical transfer of harmful, resistant substances, such as heavy metals, pesticides, mycotoxins, or pharmaceuticals, to the feed and food may take place (Charlton et al., 2015; Gold et al., 2018). Mycotoxins may even occur in EU-approved substrates not stored properly. Cadmium (Cd) and lead (Pb) accumulate in the BSFL from the rearing substrate (van der Fels-Klerx et al., 2020; Wu et al., 2020), which is different from mycotoxins (Lalander et al., 2016; Purschke et al., 2017; Bosch et al., 2017). However, in most of the few studies conducted so far, the fate of the contaminants was not followed throughout the entire food chain. Wang et al. (2017) investigated the transfer of heavy metals from swine manure to housefly larvae (*Musca domestica* L.) to chicken meat, but poultry heavy metal levels remained below the legal boundaries. However, transfer from uncontrolled common household waste could be higher (Purschke et al., 2017). Other studies (Korish and Attia, 2020; Kar and Patra, 2021) also demonstrated the transfer of heavy metals from feed to meat and eggs. In addition, mycotoxins in poultry feed have been shown to accumulate in eggs and meat but only to a small extent (Adegbeye et al., 2020; Bart et al., 2020). It remains unclear whether poultry would excrete or metabolize mycotoxins to less harmful or simply not analyzed compounds.

Therefore, our aim was to obtain evidence of the fate of contaminants along the entire food production chain when using insects as feed. Cadmium, Pb and aflatoxin B1 (AFB1) were selected for the study. The levels of these contaminants were tracked in currently unapproved and spiked rearing substrates, in the BSFL grown on them, in the hen feed containing BSFL, and in the eggs and meat (obtained after slaughter). Four hypotheses were tested: 1) The BSFL do not or only to a small extent accumulate AFB1. 2) The transfer of Cd, Pb, and AFB1 to the meat and eggs is negligible. 3) The contaminated BSFL do not affect the hens' performance. 4) The BSFL produced on non-EU approved food waste will contain low enough contaminants to prevent an above-limit contamination of meat and eggs.

2. Materials and methods

2.1. Ethics statement

The poultry experiment was conducted at the AgroVet-Strickhof research station in Lindau, Switzerland, in autumn 2020 in accordance with the Animal Protection Act and Animal Protection Ordinance laid out by the Swiss Federal Food Safety and Veterinary Office and ARRIVE guidelines (Kilkenny et al., 2010). The experimental protocol was approved by the Swiss Cantonal Veterinary Office

Table 1
Composition of the larval rearing substrates used to produce the different larval meals (g/kg dry matter).

	C_{CH} [a]	C_{IND} [b]	HM [c]	AF [d]
Raw fruit and vegetables	400	—	—	—
Brewer's grain	300	—	—	—
Pasta production side streams	300	—	—	—
Hotel food waste	—	530	530	530
Fruit rejects	—	230	230	230
Coconut fiber	—	240	240	240

[a] C_{CH} = non-spiked rearing substrate produced in Switzerland containing EU approved rearing constituents.
[b] C_{IND} = rearing substrate non-spiked substrate produced in Indonesia containing non-EU approved constituents; rearing substrate
[c] HM = rearing substrate C_{IND}, but spiked with 10 mL/kg of a solution containing 37.5 µg cadmium/mL and 375 µg lead/mL.
[d] AF = rearing substrate C_{IND}, but spiked with 10 mL/kg of a solution of 30 µg aflatoxin B1/mL.

of Zurich (ZH 221/17).

2.2. Treatment of the rearing substrates and production of defatted meals from black soldier fly larvae

For the poultry feeding experiment, four partially defatted BSFL meals originating from two BSFL production systems that differed mainly in rearing substrate composition (e.g., substrate type, blank vs. spiked) and larval postharvest processing (e.g., drying and defatting) were used. Three of the meals were produced in a pilot-scale research facility in Indonesia (BSFL conversion plant, Waste 4 Change, Puspa Agro, Sidoarjo, Indonesia). These included a control meal (C_{IND}) and two meals produced on the same substrate and additionally spiked with heavy metals (HM) and aflatoxin B1 (AF) to mimic the potential real-life contamination of food waste. The experimental BSFL substrates were based on hotel food waste (postconsumer waste, a nonapproved rearing substrate in the EU) mixed with fruit waste from a public market (Table 1). The hotel food waste mainly consisted of rice, fish, meat, and vegetables with 230 g dry matter (DM)/kg, while the fruit waste was mainly represented by watermelons with 100 g DM/kg. To ensure that the BSFL could be separated from the residue at harvest, coconut fiber (970 g DM/kg) was added to the substrate mixture, which enhanced the DM content to about 200 g/kg. In total, the mixture consisted of 530 g hotel food waste, 230 g fruit waste, and 240 g coconut fiber/kg on a dry weight basis. This material formed the C_{IND} substrate. To study a conservative scenario for the transfer of contaminants from the BSFL-rearing substrate to poultry-derived food, the rearing substrates were spiked with high contaminant concentrations. The intended contaminations of Cd and Pb in the substrate HM were 1.9 and 18.8 mg/kg DM rearing substrate, respectively. These concentrations have previously resulted in BSFL exceeding the maximum feed material contents (van der Fels-Klerx et al., 2016). A Cd and Pb solution was prepared through the dilution of Cd and Pb standard solutions (1 mg/mL each dissolved in nitric acid, Merck, Darmstadt, Germany) with deionized water to about 37.5 μg Cd/mL and 375 μg Pb/mL, respectively. These water-dissolved forms of Cd and Pb, which are not found in nature, were used as a model to increase the availability of the metals to the larvae in a worst-case scenario. Substrate AF was spiked with an AFB1 solution prepared by dissolving AFB1 (from *Aspergillus flavus*, CAS 1162–65–8, Sigma Aldrich, Darmstadt, Germany) in methanol to 30 μg/mL. For AFB1, the intended concentration was 1.5 mg/kg DM of the rearing substrate, which exceeds the maximum content for EU feed materials (0.02 mg/kg at a DM content of 880 g/kg) and those previously studied for BSFL (0.5 mg/kg) (Bosch et al., 2017). In the preliminary experiments, no negative effects of nitric acid and methanol on BSFL growth performance were observed. Directly before feeding 175 kg of the rearing substrates were put into a commercial concrete mixer. Then, over a duration of approximately 5 min, 1.75 L of the contaminant solutions were slowly sprayed onto the substrates moving with the mixer, thus ensuring a homogenous distribution. After spraying was complete, the substrates were mixed for another 5 min. Control BSFL (C_{IND}) were reared in parallel to BSFL HM on the same rearing substrate mixture after spiking the mixture with 1.75 L tap water. To avoid cross-contamination between the spiked and control-rearing substrates, the concrete mixer was cleaned with a high-pressure cleaner between batches. The larvae on substrates HM and AF were reared in two different production cycles with different batches of waste that differed slightly in composition. To account for this, the BSFL were always reared on a non-spiked substrate (C_{IND}) in parallel to the BSFL grown on each of the two spiked substrates.

The Indonesian BSFL production system was operated as described by Dortmans et al. (2021). For the first 5 days after hatching, the BSFL were reared ad libitum on poultry feed (511B, Charoen Pokphand, Bangkok, Thailand). Then, approximately 10,000 BSFL with a mean weight of 1.3–1.5 mg DM each were placed in 61 × 43 × 15 cm plastic containers containing experimental substrate. In each container, the BSFL were fed a total of 12 kg substrate over the entire rearing cycle, which was provided in three substrate portions of similar amounts, that is, 4 kg of substrate on day 0 (beginning of rearing), 4 kg on day 4 and 4 kg on day 6. On average, the temperature and humidity were 27–29 °C and 74–83%, respectively, during rearing. After 5 days and at harvest, the average BSFL weight was determined by randomly collecting 20–50 BSFL from 3 to 4 rearing containers of the same treatment, weighing them together and dividing this weight by the number of BSFL collected. After 12 (HM and C_{IND}) and 13 days (AF and C_{IND}) of feeding, the BSFL were separated from the frass (i.e., undigested feed and larval excreta) both manually and by using a vibrating sieve. The BSFL were then frozen and dried in a gas oven (CV. Tunas Karya, Yogyakarta, Indonesia) at 65 °C for 3–5 h. Afterwards, the BSFL were defatted using a heated oil press (Nanchang Dulong Industrial, Jiangxi, China). The resulting BSFL meal was dried in the same oven at 65 °C for 12 h and ground afterward using a household grinder (ZJMZYM Mini grinder, Jiangxi, China). Subsequently, the BSFL meal and fat material were sealed airtight in plastic containers and sent to Switzerland at room temperature by express via airplane transport.

To determine the effect of the substrate type, a fourth BSFL meal (C_{CH}) was produced in a research facility in Switzerland (FiBL, Frick, Switzerland). The substrate for this second control consisted exclusively of materials permitted to produce feed insects in the EU. It included food industry side stream materials and preconsumer food waste (400 g raw fruit and vegetable waste, 300 g brewer's grains, 300 g pasta production waste/kg larval rearing substrate). The production and defatting process for the C_{CH} BSFL meal was previously described in detail by Heuel et al. (2021a). Briefly, the BSFL were harvested after 14 days of growth. They were killed by freezing, followed by drying in a chamber (TSW 700 ED, Salvis Instruments, Switzerland) at 60 °C for 1.5 days. Defatting was performed using a modified commercial oil press (KK 20 F Universal, Screw Press, Reut, Germany). Subsequently, all material was frozen until used to produce the hens' diets.

Because of limitations in the experimental facilities and to ensure sufficient replicates, it was decided not to include a positive control without BSFL meal because it was assumed that the larval substrate C_{CH} did not contain significant amounts of the contaminants. However, further studies are needed to show whether the BSFL generally accumulate such contaminants from the substrate.

2.3. Experiment with laying hens

Thirty-six laying hens (Lohmann-LSL Classic) purchased at 92 weeks of age from a local poultry farm (Inauen AG, Dürnten,

Switzerland) and with an initial body weight of on average of 1.67 ± 0.15 kg were housed individually in cages with a size of 80 cm × 80 cm × 80 cm. The late stage of laying was chosen to be able to slaughter them as spent hens, thus allowing contaminant recovery in the meat, organs and eggs to be determined within the same experiment. Nine hens each were assigned to one of four experimental diets in a randomized design. These diets contained 200 g/kg dietary DM of the partially defatted BSFL meals and were formulated to cover the requirements of late-laying hens (Table 2). With the control diet C_{CH}, it was possible to investigate poultry food contamination when switching from approved larvae substrates to ones with non-approved and less controlled origins. All diets were produced at the research facility in Lindau (Switzerland). The complete diets were prepared in a single-shaft mixer with a volume of 100 kg (Gericke, Zurich, Switzerland). Diet components were mixed until the mixture was homogenous. The diets were stored at + 4 °C and allowed to adjust to room temperature 24 h before feeding. After a 1-week adaptation period (week 0) to the new housing system with conventional, soybean-based feeding, the experimental diets were fed for 4 weeks (week 1 to week 4). Feed and water were provided ad libitum with a trough and two nipple drinkers per cage, respectively. Feed was replenished twice per week or whenever more than half of the feed had been eaten. The temperature in the hen house was kept at 20 °C and the humidity at around 40–45% throughout the entire experiment. A regime of 16 h of artificial light and 8 h of dark was applied. The individual cages were equipped with a nest, a perch, and a box filled with sawdust. The health status of the animals was visually monitored daily. No hen died or showed signs of illness during the trial. At the end of the experiment, the hens were slaughtered by stunning with a blow to the head followed by exsanguination. Nine replicates and feeding diets for 4 weeks were considered sufficient to determine whether there was significant contaminant transfer to the eggs and meat. To quantitatively determine the carry-over rates, more extended studies are needed.

2.4. Data recording and sampling

In the Indonesian production unit, the fresh rearing substrates were sampled directly before feeding to the BSFL and frozen at − 18 °C (no sample of the C_{CH} rearing substrate was available). For the frass, samples from treatments C_{IND} and HM were collected at harvest from two different rearing containers. No frass samples were taken in the second production cycle. The samples of rearing substrate and frass from treatments C_{IND} and HM were dried at 105 °C for 24 h in an oven (UN55, Memmert, Schwabach, Germany). The rearing substrate AF was freeze-dried for 10 h to ensure that mycotoxins were not destroyed by oven drying. Samples of the BSFL meals produced in Indonesia and Switzerland were taken before the hen diets were formulated, and hen diet samples were taken once before the poultry experiment. All samples were ground to 0.5 mm using a centrifugal mill (model ZM1, Retsch, Haan, Germany).

The body weight of the hens was measured once a week. Feed consumption per hen was recorded twice a week by comparing the amounts offered and the amount removed from the troughs. Throughout the experiment, all eggs were collected and weighed daily. In

Table 2
Ingredient composition of the experimental laying hen diets (g/kg dry matter).

	Diet[a]			
	C_{CH}	C_{IND}	HM	AF
Defatted larval meal[b]				
C_{CH}	200	—	—	—
C_{IND}	—	200	—	—
HM	—	—	200	—
AF	—	—	—	200
Wheat	455	455	455	455
Corn	144	144	144	144
Wheat bran	40	40	40	40
Corn gluten meal	30	30	30	30
Calcium carbonate	27	27	27	27
Limestone grit	70	70	70	70
Dicalcium phosphate	10	10	10	10
Sodium bicarbonate	3.3	3.3	3.3	3.3
Sodium chloride	2.0	2.0	2.0	2.0
Choline chloride	0.8	0.8	0.8	0.8
Vitamin and trace element premix[c]	2.0	2.0	2.0	2.0
Celite[d]	16	16	16	16

[a] Diet C_{CH} = negative control diet based on non-spiked control larval meal produced in Switzerland on EU approved rearing substrates; diet C_{IND} = positive control diet based on non-spiked larval meal produced in Indonesia on non-EU approved rearing substrates; diet HM = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with heavy metals; diet AF = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with aflatoxin B1.

[b] Larval meal C_{CH} = negative control larval meal produced on non-spiked control larval substrate from Switzerland containing EU approved rearing substrates; larval meal C_{IND} = positive control larval meal produced on non-spiked larval substrate from Indonesia containing non-EU approved ingredients; larval meal HM = larval meal produced on the Indonesian rearing substrate spiked with heavy metals; larval meal AF = larval meal produced on the Indonesian rearing substrate spiked with aflatoxin B1.

[c] Provided per kg of total diet: calcium, 0.17 g; phosphorus, 0.4 g; magnesium, 0.05 g; copper, 0.1 g; manganese, 0.06 g; iodine, 0.8 mg; zinc, 0.05 g; iron, 0.05 g; selenium, 0.2 mg; vitamin A, 10,000 IU; vitamin D_3, 2500 IU; vitamin E, 0.03 g; vitamin K, 0.003 g; vitamin B_1, 0.002 g; biotin, 0.5 mg; folic acid, 1.5 mg; niacin, 0.04 g; pantothenic acid, 0.016 g.

[d] No. 545, acid-washed diatomaceous earth (Schneider Dämmtechnik, Winterthur, Switzerland).

weeks 0 (last day of the adaptation period, that is before feeding of the experimental diets), 1 (day 7) and 4 (day 28), one egg per hen of each group was cracked, and the content was frozen at − 20 °C, lyophilized for 24 h (Beta 1–16 Christ, Osterode am Harz, Germany), and ground into a homogeneous powder using a kitchen mortar (Haldenwanger, Berlin, Germany). Six eggs per hen were collected from experimental days 21–27 (week 3) to determine the general egg quality.

At slaughter, after removing the feathers, breast muscles were dissected from the carcass, trimmed of fat and skin, weighed, and immediately vacuum-sealed and stored at 4 °C. After 1 day of storage, they were homogenized in a household blender (Moulinette type DP-800, Ecully, France), frozen at − 20 °C, freeze-dried for 48 h, and ground to 0.75 mm using a centrifugal mill. In addition, the liver and kidneys of each hen were removed, weighed, and then treated like the breast meat samples, except for the fact that they were not initially homogenized.

Because only very low AFB1 levels were later found in the AF diet (see below), it was decided not to analyze eggs and body tissues for AFB1. This decision was also made because previous studies found only very low transfer rates of AFB1 from feed to poultry eggs and meat. Accordingly, the ratio of concentrations in feed to those in poultry ranged from 1111:1 (Aly and Anwer, 2009) to 5000:1 (Oliveira et al., 2000) in eggs and 1957:1 in muscles (Hussain et al., 2010). Even when taking the highest transfer rate, the expected AFB1 concentrations in eggs and meat from the present experiment would have been far below the detection limit of the method used.

2.5. Laboratory analyses

All analyses were carried out in duplicate. All types of samples were subjected to analysis according to standard procedures (AOAC International, 1997) where available. The contents of DM and total ash were analyzed using a thermogravimetric device (TGA-701, Leco, St. Joseph, MI, USA; AOAC Official Method 942.05). Organic matter was calculated as the difference between them. Nitrogen (N) in the BSFL meals and hen diets was determined using a C/N analyzer (TruMac CN, Leco, St. Joseph, MI, USA; AOAC index no. 968.06). Ether extract (EE) was measured with a Soxhlet extraction system (B-811, Büchi, Flawil, Switzerland; AOAC index no. 963.15). The chitin content was determined according to Black and Schwartz (1950). The methods used to analyze the contents of calcium, phosphorus and magnesium are described in detail in Heuel et al. (2021a). Samples of rearing substrates, frass (C_{IND}, HM), defatted larval meals (all), hen diets (all), and eggs and body tissues (C_{IND}, HM, AF) were analyzed for Cd and Pb according to the method provided by Hoenig (2001). Briefly, about 200 mg per fresh sample was extracted with 2 mL of nitric acid, followed by 10-fold dilution with distilled water. Analysis by ICP-MS (octopole reaction system, series 7500ce, Agilent, CA, USA) was then carried out.

Hen diets C_{CH} and AF, as well as the rearing substrates C_{IND}, HM and AF, and frass C_{IND} and HM, were analyzed for aflatoxin concentrations with a newly developed method. Solid, certified standard substances of AFB1 and aflatoxin M1 (AFM1) (Sigma, St. Louis, MO, USA; Aokin, Berlin, Germany) were dissolved in acetonitrile containing acetic acid (10 mL/L) and stored in the dark until further use. U-[13C17]-AFB 1 and U-[13C17]-AFM1 in acetonitrile (Biopure, Romer Labs, Tulln, Austria) were used as internal isotope standards, and phosphate-buffered saline was used to extract the immunoaffinity columns. Before analysis, samples were subjected to extraction and purification. For this purpose, 0.5 g of the sample was weighed into a 15 mL Falcon tube. Then, 0.1 g of NaCl and 5 mL of extraction solution (ACN/H_2O 80/20 v/v) were added, along with 8 mL of hexane to remove residual fat. The sample was mixed for 30 min (Turbula T2C and F, Willy A. Bachofen, Basle, Switzerland) and then centrifuged for 10 min at room temperature (Heraeus Megafuge 16, Thermo Fisher Scientific, Waltham, USA). Next, 4 mL of the organic acetonitrile phase was mixed with 46 mL of phosphate-buffered saline, and 50 μl of the internal standard was added. The extracts were purified by transferring the entire solution to the immunoaffinity columns (Aflatest, VICAM®, Watertown, MA, USA). The columns were washed twice to remove the matrix. AFB1 and AFM1 were eluted in two steps with 0.5 mL of MeOH each. Subsequently, the eluted samples were analyzed using an HPLC system (NEXERA X2 series HPLC system, Shimadzu Corporation, Kyōto, Japan) coupled with a triple quadrupole MS (QTRAP 6500 +, Sciex Germany GmbH, Darmstadt, Germany) equipped with an IonDrive™ Turbo V electrospray ionization source. The two analytes were separated using an analytical C18 Gemini NX column (100 mm × 2.0 mm, 3 μm particle size) (Phenomenex, Torrance, USA). Eluent A consisted of distilled water, 1 mL formic acid, and 300 mg/L NH4COOH/L. In eluent B, distilled water was replaced with methanol. At a flow rate of 0.5 mL/min, the proportion of eluent B linearly increased to 0.6 of the total proportion at 4.0 min after an initial time of 0.8 min with a total proportion of 0.15, reaching 0.65 of the total proportion at 6.0 min and 0.95 of the total proportion at 8.5 min. The proportion of eluent B was maintained until 9.5 min. After 10 min, the proportion of eluent B was reduced to 0.15 of the total amount. This procedure was completed within 12.5 min. The column temperature was set to 35 °C, and the injection volume was 5 μl. Mass spectrometric detection was performed in positive electrospray ionization mode, and selected reaction monitoring was used as the scan type. The values for the AFB1 content were corrected for recovery. To validate the method, blank samples of defatted insect material (C_{CH}) were spiked with 50 μl of an AFB1 and AFM1 mixture (cAFB1 = 45.5 ng/mL, cAFM1 = 42.2 ng/mL). This resulted in levels of approximately 4.55 μg/kg for AFB1 and 4.22 μg/kg for AFM1. Three additional samples, each on two more days, were spiked to determine the interday precision. The spiked samples were dried overnight. Afterwards, the limit of detection of the blank samples and limit of quantification was determined and derived according to Wenzl et al. (2016). The samples were spiked for this purpose with AFB1 and AFM1, resulting in levels of 0.45 μg/kg and 0.42 μg/kg. Repeatability (intraday precision) and recovery were calculated from each set of 10 spiked samples. Interday precision was determined using the average of three spiked samples on two days and 10 spiked samples on the third day. The linear range was derived from the calibration concentration range of seven equidistant calibration solutions. The results of the validation were as follows: LOD = 0.04 μg/kg, LOQ = 0.13 μg/kg, recovery = 96.5%, repeatability a (10 spiked samples) = 9.9%, repeatability b (3 samples on 2 days and 10 samples on the third day were spiked) = 3.1% for AFB1 and LOD = 0.06 μg/kg, LOQ = 0.22 μg/kg, recovery = 93.6%, repeatability a = 6.9%, repeatability b = 5.9% for AFM1.

Eggshell breaking strength was determined using an electronic hardness tester (type PTB 301, Pharma Test, Hainburg, Germany). The eggshell thickness was evaluated at the flat pole of the egg with a thickness gauge (Bruetsch Ruegger, Urdorf, Switzerland) after

removal of the egg membrane. The weights of whole eggs, yolks, and eggshells, as determined after cleaning from the membrane and drying at 40 °C for 24 h, were recorded. The albumen weight was calculated as the difference of egg weight minus the weights of shell and yolk. Yolk and albumen heights were measured using an analog precision dial indicator (Käfer Messuhrenfabrik, Villingen-Schwenningen, Germany). The latter was used to calculate the Haugh units (formula given by Haugh, 1937) as an indicator of egg foaming quality.

2.6. Calculations and statistical analyses

All data from the hen experiment were combined to one value per hen or feed item. Hen-related data were subjected to analysis of variance using a linear mixed model (procedure Mixed) in SAS (2010). Diet was considered as fixed effect, and hens housed individually in cages were treated as an experimental unit. Visual inspection was performed to check for normal distribution and homogeneity of variance. Comparisons among least square means were performed using the Tukey-Kramer option. The effects were considered significant at $P < 0.05$. For the calculation of the AFB1 concentrations by linear regression analysis, the areas of the peaks of samples and calibrations measured were integrated using the SCIEX (2015) MultiQuant Software.

3. Results

3.1. Contamination of the materials along the food chain with heavy metals and aflatoxin

Heavy metal spiking increased Cd and Pb concentrations in substrate HM by about 21-fold (1.5 mg/kg DM) and 18-fold (14.5 mg/kg DM), respectively, compared to the control substrate C_{IND} (0.07 mg Cd and 0.8 mg Pb/kg DM) (Table 3). These concentrations were near the targeted spiking levels of 1.8 mg Cd and 18.8 mg Pb/kg DM. Substrate AF had a higher Cd concentration (0.8 mg/kg DM) than the corresponding batch of C_{IND} (0.05 mg/kg DM). The C_{IND} and HM frass contained Cd levels similar to the corresponding substrates, whereas the Pb concentration was about three times higher than that of the corresponding substrate. Spiking of the rearing substrate with AFB1 increased its concentration by about a thousand times (842 ug/kg DM) compared to substrates C_{IND} (0.34 µg/kg DM) and HM (0.62 µg/kg DM). Still, this AFB1 level in substrate AF was lower than the level intended (1500 µg AFB1/kg DM). Because of the scarcity of samples, AFB1 could not be analyzed in the AF frass. However, for C_{IND} and HM frass, the AFB1 concentration was similar to those levels determined in the corresponding substrates (proportionately −0.26 for C_{IND} and +0.03 for HM) (Table 3). Aflatoxin M1 was below the detection limit in all substrate and frass samples (data not shown in the table).

Corresponding to the substrates, the defatted BSFL meal HM had the highest level of Cd and Pb, and in BSFL meal AF, AFB1 was detected. The BSFL meals C_{CH} and C_{IND} showed a similar level of Cd (0.2 mg/kg), but the Pb level was about 3 times higher in BSFL meal C_{IND} compared with BSFL meal C_{CH}. Lead concentration in the BSFL meal AF was comparable to that found in BSFL meal C_{IND}, while the Cd concentration was eight times greater. The contaminant levels analyzed in the complete hen diets broadly corresponded with BSFL meal inclusion levels and contaminant concentrations.

3.2. Effects of spiking with heavy metals and aflatoxin B1 on larval development

Heavy metal spiking of the substrate did not alter BSFL development. After 5 days and at harvest (12 days for HM, 12 and 13 days for C_{IND}, 13 days for AF), individual weight was 90 ± 12 mg and 309 ± 46 mg for C_{IND} BSFL and 84 ± 10 mg and 352 ± 52 for HM BSFL, respectively. In contrast, spiking of the substrate with AFB1 impaired BSFL development. After 5 days, the individual BSFL weight was 77 ± 15 mg with substrate AF and 112 ± 9 mg with substrate C_{IND}. Additionally, at harvest, unlike AF larvae, many of the C_{IND} BSFL had already entered the prepupal stage, indicating faster development. The weight loss occurring toward the prepupal stage explains the lower weight of the C_{IND} BSFL found at harvest of 253 ± 19 mg compared with 312 ± 8 mg of AF BSFL.

3.3. Nutrients in BSFL meals and diets as well as hen performance and egg quality

BSFL meal AF was the highest in N and chitin (Table 4). Larval meals C_{CH} and AF were higher in EE than BSFL meals C_{IND} and HM. The BSFL meal C_{CH} contained much less calcium and magnesium than the BSFL meal from Indonesian production. Because the hen

Table 3
Measured concentrations of the contaminants in the rearing substrates and frass of the larvae production per kg of dry matter (n = 2 per treatment).

	Rearing substrate[a]				Frass[b]	
	Batch 1		Batch 2			
Item	C_{IND}	HM	C_{IND}	AF	C_{IND}	HM
Cadmium (mg)	0.07	1.54	0.05	0.80	0.03	0.91
Lead (mg)	0.79	14.74	0.34	0.35	2.20	44.72
Aflatoxin B1 (µg)	1.00	0.62	0.82	842	0.74	0.64

[a] C_{IND} = non-spiked rearing substrate produced in Indonesia containing non-EU approved constituents; HM = Indonesian rearing substrate spiked with heavy metals; AF = Indonesian rearing substrate spiked with aflatoxin B1.

[b] Complete amount remaining after the larvae had been manually collected from larvae fed either rearing substrate C_{IND} or HM. No frass of larvae from rearing substrate AF was available.

diets were composed of the same ingredients included in the same proportions, the variations in the analyzed nutrient composition of the diets reflected, to a proportionately smaller extent, the analyzed differences found in the BSFL meals in most nutrients.

Performance and intake parameters measured (feed intake and conversion rate, as well as laying performance and egg weight) were not affected by dietary treatment, except for the daily intake of Cd and Pb ($P < 0.001$; Table 5). For egg quality, only shell stability, as measured by breaking strength, was affected. Shell stability was lower ($P < 0.05$) for the C_{IND} hens compared to the HM hens by about 10 Newton, with the other groups being intermediate. However, no differences in shell thickness were found.

3.4. *Effects of BSFL meals and diets on the heavy metal concentrations of tissues and eggs*

Compared with the two control diets (C_{CH} and C_{IND}), the Cd concentrations in the liver ($P < 0.001$), kidneys ($P = 0.004$), and breast meat ($P < 0.001$) were increased almost two-fold in hens from the HM treatment (Table 6). Cd was not detected in the eggs of birds from any treatment. In the kidneys, Pb was higher ($P < 0.001$) in hens on the HM diet compared to those of the two control diets. Pb was below or at the detection limit in the breast meat and eggs and the livers, respectively. When detected, the concentrations of Cd and Pb did not significantly differ between the two control hen groups (C_{CH} and C_{IND}).

4. Discussion

4.1. *Transfer of contaminants from rearing substrates to larvae and implications for feed safety*

The sustainable production and use of BSFL as an alternative feed ingredient requires rearing substrates not directly used as food and feed. Among these, postconsumer waste may have the largest environmental benefits (Smetana et al., 2019). However, these substrates are not approved for this purpose in the EU and may contain contaminants in amounts exceeding the maximum content specified in the current feed regulations (van der Fels-Klerx et al., 2018).

In the present study, the fate of important contaminants along the food chain was investigated inclusive of spiking with contaminants. Previous studies used substrates containing 0.09–80 mg Cd/kg DM and 0.07–143 mg Pb/kg DM (summarized by Lievens et al., 2021) compared to 1.5 mg Cd/kg DM and 14.8 mg Pb/kg DM in the spiked substrates of the current study. Maximum AFB1 contents in the substrate of previous studies (Bosch et al., 2017; Purschke et al., 2017) ranged from 200 to 500 μg/kg DM compared to 842 μg/kg DM in the present study.

Insects are defined as livestock in the EU. Therefore, the maximum contents specified by the EC (Directive 2002/32/EC) for feed material of animal origin and those for complete feeds (2 and 0.5 mg Cd/kg, 10 and 5 mg Pb/kg, 50 and 20 μg AFB1/kg, respectively, at a DM content of 880 g/kg) were applied to classify the substrates and BSFL materials of the present study. The food waste not spiked with contaminants and the resulting BSFL meals did not exceed these maximum levels. Unexpectedly, the BSFL meal produced with the rearing substrate spiked with AFB1 contained Cd at a level of also 1.9 mg/kg DM, which was much higher than the corresponding control meal but still below the maximal content for feeds. This illustrates the variability which exists in food waste, and as such contaminant levels may approach or exceed the maximum EC levels.

Larval meal produced from substrate spiked with Cd and Pb, exceeded the maximum allowable EC levels for these heavy metals, consistent with results of several previous studies (Table 7). This shows that BSFL accumulate heavy metals at correspondingly high levels in the rearing substrate. The comparison with other studies is limited as we did not analyze a full-fat but a partially defatted BSFL

Table 4
Measured composition of the four defatted larval meals and the four experimental diets per kg dry matter.

	Defatted larval meal[a]				Diet[b]			
Item	C_{CH}	C_{IND}	HM	AF	C_{CH}	C_{IND}	HM	AF
Dry matter (g)	937	954	955	966	902	897	900	908
Organic matter (g)	884	778	761	793	755	755	729	720
Nitrogen (g)	79.9	63.1	62.0	97.0	34.5	30.4	31.9	31.0
Ether extract (g)	299	199	138	273	75.5	49.5	42.9	63.8
Chitin (g)	69.5	58.9	60.1	80.3	15.2	14.7	14.6	15.5
Calcium (g)	9.5	58.1	67.5	54.6	34.9	37.1	48.9	49.5
Phosphorus (g)	6.75	6.52	6.43	4.74	6.52	6.32	6.47	6.13
Magnesium (g)	0.55	1.77	2.07	1.93	12.6	13.7	15.1	13.5
Cadmium (mg)	0.22	0.24	7.08	1.86	0.18	0.19	1.68	0.61
Lead (mg)	0.23	0.75	15.5	0.48	0.07	0.16	3.20	0.18
Aflatoxin B1 (μg)	n.a.	n.a.	n.a.	4.03	n.d.	n.a.	n.a.	1.22

n.d., not detected; n.a. not analyzed.

[a] Larval meal C_{CH} = meal produced in Switzerland on EU approved substrates; larval meal C_{IND} = meal produced on non-EU approved Indonesian rearing substrate; larval meal HM = Indonesian rearing substrate spiked heavy metals; larval meal AF = Indonesian rearing substrate spiked with aflatoxin B1.

[b] Diet C_{CH} = negative control diet based on non-spiked control larval meal produced in Switzerland on EU approved rearing substrates; diet C_{IND} = positive control diet based on non-spiked larval meal produced in Indonesia on non-EU approved rearing substrates; diet HM = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with heavy metals; diet AF = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with aflatoxin B1.

Table 5
Performance of the laying hens fed the experimental diets for 28 days and the quality of their eggs (n = 9 per treatment).

Diet[a]	C_{CH}	C_{IND}	HM	AF	SEM	P-value
Daily intake						
Total feed (g/day)	108	105	101	112	3.5	0.203
Cadmium (mg/day)	0.019[c]	0.020[c]	0.170[a]	0.068[b]	0.0033	< 0.001
Lead (mg/day)	0.008[b]	0.017[b]	0.324[a]	0.020[b]	0.0061	< 0.001
Aflatoxin B1 (μg/day)	n.d.	n.d.	n.d.	0.136	—	—
Body weight at day 28 (kg)	1.66	1.60	1.68	1.69	0.040	0.461
Laying performance (%)	84.3	82.4	80.5	85.1	4.45	0.886
Egg weight (g)	62.5	63.1	63.6	65.5	1.15	0.294
Egg mass (g/day)	52.5	51.8	50.9	55.6	2.62	0.618
Feed conversion rate (g feed/g egg)	2.09	2.03	2.06	2.05	0.126	0.991
Egg quality						
Shell breaking strength (N)	33.1[ab]	25.7[b]	35.4[a]	29.3[ab]	2.51	0.049
Shell thickness (mm)	0.38	0.38	0.36	0.36	0.009	0.148
Egg composition (g/kg)						
Yolk	300	290	293	305	7.1	0.463
Albumen	553	554	546	549	8.1	0.886
Shell	147	156	161	146	5.2	0.140
Haugh units	86.9	84.9	86.4	87.4	2.09	0.863
Yolk height (mm)	18.8	18.9	19.0	18.4	0.27	0.405

n.d., not detected.
[a-b]Mean values in rows with unequal superscripts differ significantly (Tukey test, $P < 0.05$).
[a] Diet C_{CH} = negative control diet based on non-spiked control larval meal produced in Switzerland on EU approved rearing substrates; diet C_{IND} = positive control diet based on non-spiked larval meal produced in Indonesia on non-EU approved rearing substrates; diet HM = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with heavy metals; diet AF = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with aflatoxin B1.

Table 6
Measured concentrations of cadmium and lead in livers, kidneys, eggs (collected on day 28), and breast meat per kg dry matter (n = 9 per treatment).[a,b].

Diet[c]	C_{CH}	C_{IND}	HM	SEM	P-value
Cadmium[d]					
Liver (mg)	0.76[b]	0.92[b]	1.86[a]	0.158	< 0.001
Kidneys (mg)	7.13[b]*	7.69[b]*	12.29[a]*	1.056	0.004
Breast meat (μg)	6.22[b]	6.08[b]	13.34[a]	1.27	< 0.001
Eggs (μg)	n.d.	n.d.	n.d.	—	—
Lead					
Liver (mg)	n.d.	0.02	n.d.	0.007	0.180
Kidneys (mg)	0.02[b]	0.07[b]	0.49[a]	0.039	< 0.001
Breast meat (μg)	n.d.	n.d.	n.d.	—	—
Eggs (μg)	n.d.	n.d.	n.d.	—	—

n.d., not detected.
[a-b]Mean values in rows with unequal superscripts differ significantly (Tukey test, $P < 0.05$).
[a] Cadmium in the eggs (collected on the last day of the adaption period and on day 7 and 28 of the experiment) as well as lead in eggs and breast meat were not detected. Tissues from diet AF were not analyzed for heavy metals as the spiking the diet with aflatoxin B1 did not change heavy metal load compared to diet C_{IND}.
[b] Dry matter content of the fresh meat was about 250 g/kg and that of the organ samples about 300 g/kg.
[c] Diet C_{CH} = negative control diet based on non-spiked control larval meal produced in Switzerland on EU approved rearing substrates; diet C_{IND} = positive control diet based on non-spiked larval meal produced in Indonesia on non-EU approved rearing substrates; diet HM = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with heavy metals; diet AF = diet based on meal of larvae grown on the Indonesian rearing substrate spiked with aflatoxin B1.
[d] Values marked with * exceed permitted levels on wet weight basis in the EU (liver, 0.5 mg/kg).

meal, where the water-soluble heavy metals should have been increased in concentration. However, the variation among studies was much larger than that likely caused by defatting. Similar to previous studies (Table 7), Cd in frass from Cd-contaminated rearing substrates was close to the substrate concentration. In contrast, frass from the Pb-contaminated rearing substrates was about 3-fold higher in Pb concentration than in the substrate. Van der Fels-Klerx et al. (2016) observed a 4-fold higher increase in Pb concentration in frass compared to the substrate. In contrast, Purschke et al. (2017) and Diener et al. (2015) reported only a slight increase. This shows that elevation in Pb in the frass needs to be closely monitored because excessive levels may limit its use as a soil conditioner or fertilizer (Gold et al., 2018).

To the best of authors knowledge, the present study is the first to detect AFB1 in BSFL after feeding an AFB1-contaminated substrate. Still, the corresponding BSFL meal level was low at only 4 μg/kg DM and well below the EC threshold. The high level of 842 μg

Table 7
Concentrations (mg/kg dry matter) of cadmium and lead measured in the rearing substrate, the black soldier fly larval material and the frass from various studies where the rearing substrate was spiked with these heavy metals.

	Substrate		Larval material[a]		Frass		
	Cd	Pb	Cd	Pb	Cd	Pb	Substrate used
Present study	1.5	14.7	7.1	15.5	0.9	44.7	Post-consumer waste[b]
Purschke et al. (2017)	1.5	15.2	13.7	35.6	1.8	19.8	Corn semolina[c]
van der Fels-Klerx et al. (2016)	1.0	—	7.0	—	1.0	-	Chicken feed[c]
van der Fels-Klerx et al. (2016)	—	9.6	—	11.7	—	41.5	Chicken feed[c]
Diener et al. (2015)	2.7	—	7.0	-	2.9	—	Chicken feed[c]
Diener et al. (2015)	—	34.3	—	22.8	—	53.3	Chicken feed[c]

[a] Larval material in the present study was a defatted larval meal, while the other studies were based on freeze dried and ground whole larvae..
[b] Not yet approved by the EU as a rearing substrate for insects as feed.
[c] EU-approved rearing materials for insects as feed.

AFB1/kg substrate DM, compared to only 13 µg and 415 µg AFB1/kg applied by Purschke et al. (2017) and Bosch et al. (2017), respectively, possibly facilitated the detection of AFB1 in the BSFL in the present study. The authors not detecting AFB1 in the BSFL had assumed that BSFL probably incorporate mycotoxins, but that these are degraded through the NADPH-dependent enzyme AFB1 aldehyde reductase into their derivatives. This phenomenon is known from the navel orangeworm (*Amyelois transitella*) (Lee and Campbell, 2000). AFB1 metabolites, for which no analytical protocols existed for the matrices to be analyzed, were not determined in the present study. However, these metabolites appear to be less toxic than the parent compound (Meijer et al., 2019; Gützkow et al., 2021). Still, conclusive statements about the toxicity of the AFB1 metabolites in insects are limited. Another type of aflatoxin, AFM1, was not detected in substrate and BSFL meal. It remains to be clarified whether the AFB1 found in the present study originated partly, mainly, or completely from the substrate residues in the guts of the larvae. In that case, it would be advantageous to fast the insects for some time before harvest. Alternatively, a different substrate for the finishing phase of BSFL production could be used; however, the toxin risk has to be carefully considered owing to the high variability of fungal species in larval substrates (Boccazzi et al., 2017). Even the defatting process which is common in commercial BSFL production for feeding purpose might be an important step to limit the level of fat-soluble aflatoxins in the hen diets.

Concerning the complete hen diets, the EC maximum levels specified for complete feed have to be applied. The AFB1 level given refers to complete feed for adult poultry (excluding young poultry). In the present study, an inclusion level of 200 g BSFL meal/kg DM diet was tested. It is likely that a few feed producers would use higher proportions. Accordingly, Pb remained below the maximum content (5 mg/kg) in all the diets, except when including the BSFL meal produced on the heavy metal spiked substrate. Similarly, the dilution through additional feed ingredients resulted in 1.2 µg of AFB1/kg DM in the complete hen diet, which is only 0.06 of the maximum EC content (20 µg/kg). This, and all previous studies (Bosch et al., 2017; Purschke et al., 2017) indicate that BSFL material might be safe for feeding to poultry even at high AFB1 levels in the rearing substrate. This is very promising because it could justify the use of nutrient-rich but AFB1-contaminated wastes and feed materials for animal feed production via BSFL. However, to confirm this the rearing substrates have to be analyzed for mycotoxins other than AFB1 and AFM1. Furthermore, to establish a safe level for rearing substrates, more information about the exact metabolism of AFB1 in BSFL and the toxicity of the metabolites would be needed. In insect cultivation under warm and humid condition, further substrate contamination by various mycotoxins formed by storage molds may occur.

4.2. Influence of spiking on larval and hen performance and on general egg quality

An important aspect for the efficiency of BSFL production as feed is their growth rate. Like other animals, BSFL might suffer from toxicity when consuming contaminated substrates. Indeed, Diener et al. (2015) showed that substrates containing 5.9–142 mg/kg Pb impaired growth and development of the BSFL. We did not use such a highly Pb contaminated rearing substrate as Diener et al. (2015) and did not detect growth reduction when spiking the substrate with Pb and Cd. In contrast, the spiking of the substrate with AFB1 turned out to be much more critical because it clearly impaired the development of the BSFL in the present study.

A decrease in hen performance or egg quality would be similarly disadvantageous when including BSFL, especially those grown on contaminated substrates. Palatability of a diet including 150 g BSFL meal/kg DM was found to be high by Heuel et al. (2021a) when compared to a diet based on soybean meal as protein source. Also at 200 g BSFL meal/kg, the feed intake was in the normal range for laying hens in the present study. In the present study, feed intake was unaffected by treatment. Performance was also at a high level (80–85%) for aged layers suggesting nutrient supply across the diets was adequate in the present study. Marono et al. (2017) and Heuel et al. (2021a) also reported that the performance of laying hens was unaffected when fed BSFL diets. Spiking the rearing substrate had no adverse effects on intake, performance, or general egg quality. The observed differences in shell stability are most likely due to individual variations at this age of the hens and not a consequence of the different diets or their contaminant loads. High levels of heavy metals in poultry diets may impair performance. Broilers fed diets with either 600–2400 mg Pb/kg (Wittmann et al., 1994) or 540 mg Pb/L (via 200 g/kg battery waste; Alagbe, 2016) exhibited a growth depression. However, these levels are much higher than the 3.2 mg Pb/kg of the HM diet used in our study. In order to further minimize adverse effects of Pb-contaminated larval material, offering the choice of a second, low-PB, diet could be helpful as detrimental levels would then be actively avoided by selecting sufficient low-PB

feed (Wittmann et al., 1994). For Cd, Olgun and Bahtiyarca (2015) reported an adverse effect of the heavy metal on the performance of laying hens. However, this only occurred when dietary Cd was at 45 mg /kg, which far exceeds the 1.7 mg Cd/kg of diet HM in our study. The low AFB1 level in the AF diet in our study was not expected to, and did not, adversely affect hen performance.

4.3. Contaminant levels in eggs and meat from laying hens fed diets with larvae grown on contaminated substrates

The small part of the heavy metals ingested by livestock with feed, which is not excreted, are deposited mainly in the kidneys and liver (Mohammed et al., 2013; Korish and Attia, 2020). Still, excessive contaminant intake can lead to a noticeable transfer into meat and eggs, which can render the products unusable as food. The maximum levels set by the EC (EC No. 1881/2006) for Cd in meat, liver, and kidneys are 0.05, 0.5, and 0.5 mg/kg (wet weight basis). The corresponding maximum level of Pb in meat is 0.1 mg/kg. For liver and kidneys, the level of 0.5 mg/kg for slaughtering by-products (including organs) applies. If the maximum levels are exceeded, only the corresponding tissue is not marketable. Therefore, the meat can be used as food even when livers or kidneys have to be excluded from human consumption. Despite not exceeding the maximum Cd levels in the hen diets C_{CH}, C_{IND}, and HM, the measured Cd levels ranging from 2.1 to 3.7 mg/kg hens' kidneys exceeded the corresponding thresholds. For the livers, only spiking elevated Cd above the maximum levels set by the EC, and this to a slight degree. The breast meat of all groups contained very low Cd levels (3–6 µg Cd/kg). The same applied to Pb in the liver, kidneys, meat and eggs of all three analyzed groups. There are only a few reports on heavy metal enrichment of poultry-derived foods in the literature and, to the best of the authors' knowledge, no studies were conducted with specifically contaminated feed ingredients. Bokori et al. (1995) demonstrated that, at a level of 75 mg/kg diet, Cd concentration in quail eggs increased from the third day of feeding, up to 0.78 mg Cd/kg egg. The Cd in the eggs peaked in week 2 of feeding then substantially decreased. The authors explained these findings by a possible reduction of the protein concentration needed for the transport and absorption of Cd at high Cd exposure and a concomitant decrease in the excretory activity in the oviduct of the birds. Elsharawy and Elsharawy (2015) detected both Cd and Pb in chicken breast meat and organs obtained from conventional slaughterhouses but at levels that did not exceed the maximum EC levels. No dietary information was given in these publications. Conversely, Korish and Attia (2015) did not detect Cd and Pb in the meat and organs of broilers fed conventional feeds. The high Cd levels in the kidneys of the hens across all diet groups in our study could be due accumulation with age of the hens. For example, Vos et al. (1990) showed that Cd in the kidneys of turkeys increased age from initially 0.04–0.15 mg/kg after 12 weeks and to 0.6 mg/kg in the spent hens. In addition, at certain locations there may be Cd exposure via air or soil pollution (Jevsnik and Doganoc, 2003; Kar et al., 2018).

In the present study, the contamination of the hen diet with AFB1 was far below the maximum level permitted by EC; several studies showed that AFB1 transfer from feed to meat and eggs is very low. Oliveira et al. (2000) detected 0.1 µg AFB1/kg eggs in 20 week-old laying hens fed a diet of 500 µg AFB1/kg for 8 weeks, whereas no AFB1 was detected at lower dietary levels. Zaghini et al. (2005) found no AFB1 or AFM1 in eggs when feeding a diet with 2500 µg AFB1/kg for 4 weeks to 44 weeks old laying hens and found very low levels in the liver (< 5 µg/kg). Pandey and Chauhan (2007) added 0, 2500, 3130 and 3900 µg/kg AFB1 to the diet of laying hens (1–40 weeks of age). After 280 days of feeding they detected AFB1 in the eggs and in the breast meat of all AFB1 test groups. The highest load in the feed of that study led to 1.63 and 25.7 µg AFB1/kg eggs and breast meat, respectively. The authors explained this high enrichment by the long duration of feeding. The significantly lower contamination of our feed with AFB1 and the short feeding duration justifies the decision not to analyze for AFB1 in the hens‘ tissues and eggs in the present study.

4.4. Study limitations

The present findings only apply for the conditions of 4 weeks of feeding contaminated larval material. It can be expected that accumulation of contaminants may increase with longer-term feeding to layers. This length of the study, however, gives valuable indications on the contamination of meat and organs to be expected with broilers. Examining the contaminant levels of the hens would have shown whether they had already accumulated the contaminants investigated before the start of treatment feeding and thus have enabled to assess the real rate of accumulation of the heavy metals which is crucial and might rule out insect meals with even moderate levels of heavy metals and other potentially dangerous contaminants. Future studies therefore should quantitatively follow the transfer of the contaminants through the food chain to determine transfer factors, such as already determined for the last step from feed to animal-source food in the review of Leeman et al. (2007). Finally, further studies should include an insect-free control group in experiments with poultry.

5. Conclusion

Major contaminants were traced from black soldier fly larval rearing substrate to the larval meal and to the eggs and meat of laying hens. The transfer of heavy metals from larvae to egg and meat was low to negligible, whereas cadmium in the kidneys exceeded the maximum EC levels, independent of spiking. Fasting before harvest might reduce larval heavy metal contamination by emptying contaminants from their digestive tract. Different from the heavy metals, levels of aflatoxin B1 were low even in the larvae. However, the accumulation of potentially harmful metabolites cannot be excluded. In addition larval growth depression with a high addition of aflatoxin is a drawback. Because hen performance and egg quality were not affected, food waste could be a suitable rearing substrate for black soldier fly larvae and fed to meat and egg producing poultry, but for laying hens this still needs to be demonstrated in a long-term study.

CRediT authorship contribution statement

M. Heuel: Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft, Writing – review & editing. **M. Kreuzer**: Project administration, Conceptualization, Resources, Funding acquisition, Methodology, Validation, Supervision, Writing – review & editing. **I. D. M. Gangnat**: Conceptualization, Methodology, Writing – review & editing. **E. Frossard**: Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing. **C. Zurbrügg**: Conceptualization, Methodology, Resources, Funding acquisition, Writing – review & editing. **J. Egger**: Investigation, Methodology, Resources, Writing – review & editing. **B. Dortmans**: Investigation, Methodology, Resources, Writing – review & editing. **M. Gold**: Conceptualization, Methodology, Resources, Writing – Review & editing. **A. Mathys**: Conceptualization, Methodology, Funding acquisition, Writing – review & editing. **J. Jaster-Keller**: Formal analysis, Methodology, Resources, Writing – review & editing. **S. Weigel**: Formal analysis, Methodology, Resources, Writing – review & editing. **C. Sandrock**: Conceptualization, Methodology, Funding acquisition, Resources, Writing – review & editing.**M. Terranova**: Conceptualization, Methodology, Resources, Validation, Supervision, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors report no conflict of interest.

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