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# Tracing the journey of elements from fish feed to Nile tilapia faeces to black soldier fly larvae: a comparative approach

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**Introduction:** The circular bioeconomy concept revolves around biological production cycles that reintroduce products or waste from one production system to another, aiming to maximize resource utilization while minimizing environmental impact. The purpose of this study was to evaluate and compare the element flow when integrating black soldier fly larvae (BSF) production with Nile tilapia production using varying experimental fish feed.

**Methods:** Tilapia ( $42.5 \pm 11.2$  g) were reared in recirculating aquaculture systems (RAS) at 25.5°C for 10 weeks and fed equal daily rations of four experimental diets containing fishmeal (FM), poultry blood meal (PBM), black soldier fly meal (BSF) and poultry by-product meal (PM) as the single main protein source, respectively. Faeces was collected daily from settling columns installed in the RAS and subsequently fed to BSF larvae.

**Results and discussion:** The fish exhibited the highest biomass gain when fed with FM (1,001 g) or PM (901 g). The growth was lowest for those fed with PBM (406 g). The fish fed with PBM also produced the highest amount of faeces (234 g). When the fish faeces were utilized as a substrate for rearing black soldier fly (BSF) larvae and although the biomass gain did not differ significantly, the feed conversion ratio (FCR) varied among larvae fed with different fish faeces, ranging from 8.36 to 25.04. Furthermore, the concentration of analysed elements (Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, and Zn) varied based on the type of fish faeces provided. Results illustrate that a modulation of BSFL elemental composition is possible through manipulating the feed provided to the fish and emphasize the importance of fish feed composition for fish performance as well as the compositional quality of the larvae reared on the resulting fish faeces. Hence, if BSF larvae production is to be harnessed for the recycling of aquaculture sludge, ingredient choice and subsequent elemental composition of fish feeds are pivotal to larvae composition.

## KEYWORDS

black soldier fly, fish faeces, waste management, fish feed, insect protein

# 1 Introduction

The circular bioeconomy concept embraces maximal utilization of biological resources, thereby addressing sustainability challenges and fulfilling societal demands. This approach considers biological production cycles that reintroduce products or wastes from a production system to the other in order to achieve maximal use of resources and minimal environmental impact (Zaman et al., 2023). Over the past years, the European Union has been actively promoting the responsible management and, when possible, the utilization of biowastes (Fava et al., 2015). This includes the appropriate processing of raw materials and biological resources, as well as the protection and conservation of environmental media such as water and soil (Schepelmann et al., 2006; Vehlow et al., 2007; Fava et al., 2015). These developments gave rise to “waste to value” business models that integrate different biorefinery techniques and biowaste processing methods to create value from wastes (Zaman et al., 2023).

The mass-rearing of insects has gained worldwide attention in recent years as a sustainable and efficient means of waste treatment and protein production (Gasco et al., 2020; Raksasat et al., 2020). This process offers several advantages over traditional waste treatment methods, including reduced environmental impact, resource recovery, and the production of valuable by-products (Salomone et al., 2017; Ojha et al., 2020; Sangiorgio et al., 2022).

The black soldier fly (*Hermetia illucens*) is one of the most commonly mass-reared insect species (Tömberlin and Van Huis, 2020). The larvae have a voracious appetite and can consume and thrive on different organic waste streams (Raksasat et al., 2020), including fruit and vegetable wastes (Borel et al., 2021), catering wastes (Candian et al., 2023), agricultural wastes (Beyers et al., 2023; Yakti et al., 2023), and human and animal manure (Sheppard et al., 1994; Diener et al., 2011; Lalander et al., 2013). The larvae efficiently convert organic substrates into a protein-rich larval biomass, which can be harvested and used as a sustainable feed component (Gasco et al., 2020). The bioconversion process also generates valuable by-products such as the rest substrate, also known as frass, which can be used as a plant fertiliser (Beesigamukama et al., 2020).

In aquaculture, the black soldier fly larvae (BSFL) meal has been shown to enhance the nutrition and health of aquatic organisms. The incorporation of BSFL in the feed of Nile tilapia, for example, has led to improved growth, enhanced feed efficiency, and better development of liver and intestinal organs (Abdel-Tawwab et al., 2020; Limbu et al., 2022). Positive effects were also observed in other fish species such as rainbow trout (*Oncorhynchus mykiss*) (Elia et al., 2018), Atlantic salmon (*Salmo salar*) (Belghit et al., 2018), and Jian carp (*Cyprinus carpio*) (Li et al., 2017).

Generally, aquaculture produces wastes that can be categorized as solid or dissolved wastes. Solid wastes primarily consist of fecal droppings, unconsumed feed, and occasionally, fish carcasses when mortalities occur (Akinwale et al., 2016). The presence of solid wastes in the aquaculture system causes issues such as boosting aerobic bacterial activity leading to the depletion of oxygen and the reduction of biofilm formation (Michaud et al., 2006), ultimately reducing the biofilter efficiency and leading to the accumulation of fish-toxic ammonia. The adverse effects of elevated ammonia levels include decreased feed intake, reduced growth rates, vulnerability to diseases, and disruption of various physiological processes (Levit, 2010). In well-managed farms, approximately 30 percent of the feed given is

expected to remain in the system as solid wastes, although this percentage may vary based on the system operation (Miller and Semmens, 2002). The use of recirculating aquaculture systems (RAS), for example, is gaining interest as they enable better solid waste removal compared to conventional flow-through systems (d'Orbcastel et al., 2009).

In theory, if black soldier fly larvae (BSFL) can consume waste materials obtained from aquaculture tanks, (i.e., fish faeces and feed leftovers), it would be possible to establish a feedback loop by incorporating BSFL into aquafeed. This feedback loop would confer an extra environmental advantage by reducing the pollution caused by aquaculture systems and generating value from waste.

However, currently the utilization of fish wastes as a substrate for black soldier fly larvae (BSFL) is prohibited in the European Union (EU) due to the associated risks related to the use of animal manure as a rearing substrate. Given that insects are farmed animals, their rearing should comply with legal frameworks like those established by the European Union (Commission Regulation (EU) 2017/893), and their feeding substrate should meet specific requirements. Aside from the legal issues with using fish faeces as a rearing substrate, the maximum allowed levels of heavy metals and other contaminants must not be exceeded in insects used for feed production.

Few studies have assessed the potential of utilising aquaculture sludge as feeding substrates for BSFL (Schmitt et al., 2019; Liland et al., 2023; Zhang et al., 2023). However, the properties and composition of fish faeces collected from aquaculture farms can hypothetically vary based on many factors such as the fish species and the feed provided leading to a variance in the composition of produced BSFL not considered in the above studies. We hypothesize that the composition of the fish feed, as the starting point of the nutrient flow, will influence the growth and elemental composition of the resulting BSFL. Furthermore, we anticipate that the accumulation of elements in the BSFL will correlate with the initial composition of the BSFL substrate, i.e., the fish faeces. To test these hypotheses, we coupled BSFL rearing to Nile tilapia production in RAS systems. The fish received four experimental feeds differing in their protein sources, i.e., fish meal (FM), black soldier fly larvae meal (BSFM), poultry blood meal (PBM), and poultry by-product meal (PM). The various types of fish faeces obtained were used as feeding substrates for BSFL, and their performance and composition were compared.

## 2 Materials and methods

### 2.1 Fish feeding trial

#### 2.1.1 Experimental diets

Four experimental diets were formulated to be isonitrogenous (~40% crude protein) and isolipidic (~12% crude fat). The diets were formulated with fish meal (FM), black soldier fly larvae meal (BSFM), poultry blood meal (PBM), and poultry by-product meal (PM) as the single main protein source. BSFM, PBM, and PM have been used to replace FM in fish diets (Galkanda-Arachchige et al., 2020; Alfiko et al., 2022; Shaw et al., 2022a) and are commercially available as fish feed components. Diets were extruded at SPAROS I&D, Olhão, Portugal, (SPAROS) and stored at -20°C until use. Diet formulations and proximate composition are given in Table 1, and the mineral composition is given in Table 2.

TABLE 1 Fish diet formulation and proximate composition.

|  | Experimental Diets |              |              |              |
|--|--------------------|--------------|--------------|--------------|
|  | FM                 | BSFM         | PBM          | PM           |
| <b>Ingredient composition (% incorporation)</b>      |                    |              |              |              |
| Fish meal <sup>1</sup>                               | 51.0               | –            | –            | –            |
| Black soldier fly larvae meal <sup>2</sup>           | –                  | 61.6         | –            | –            |
| Poultry blood meal <sup>3</sup>                      | –                  | –            | 37.2         | –            |
| Poultry meal <sup>4</sup>                            | –                  | –            | –            | 56.4         |
| Wheat bran <sup>5</sup>                              | 29.8               | 19.9         | 39.4         | 26.0         |
| Corn meal <sup>6</sup>                               | 11.0               | 11.0         | 11.0         | 11.0         |
| Vitamin and mineral premix <sup>7</sup>              | 1.0                | 1.0          | 1.0          | 1.0          |
| Dicalcium phosphate (DCP) <sup>8</sup>               | 1.2                | 1.2          | 1.2          | 1.2          |
| Fish oil <sup>9</sup>                                | 3.0                | 3.0          | 3.0          | 3.0          |
| Poultry fat <sup>10</sup>                            | 3.0                | 2.3          | 7.2          | 1.4          |
| <b>Proximate composition (%—as fed)<sup>11</sup></b> |                    |              |              |              |
| Dry matter (DM)                                      | 91.90 ± 0.10       | 92.30 ± 0.10 | 91.60 ± 0.10 | 93.05 ± 0.15 |
| Crude fat (CF)                                       | 11.55 ± 0.15       | 11.10 ± 0.20 | 11.60 ± 0.10 | 11.85 ± 0.25 |
| Crude fibre (CFB)                                    | 2.85 ± 0.05        | 8.05 ± 0.15  | 4.20 ± 0.00  | 3.10 ± 0.00  |
| Ash  | 11.65 ± 0.05       | 8.25 ± 0.05  | 4.35 ± 0.05  | 10.85 ± 0.05 |
| Starch   | 12.05 ± 0.45       | 12.65 ± 0.25 | 13.45 ± 0.05 | 9.00 ± 1.00  |
| Nitrogen-free extract (NFE) <sup>12</sup>            | 25.55 ± 0.05       | 24.55 ± 0.55 | 30.55 ± 0.35 | 23.55 ± 0.45 |
| Gross energy (GE) (MJ/kg) <sup>13</sup>              | 18.48 ± 0.03       | 18.14 ± 0.00 | 19.49 ± 0.00 | 19.05 ± 0.05 |
| Crude protein (CP) (% - N xS 6.25)                   | 42.3 ± 1.10        | 43.8 ± 0.03  | 43.47 ± 1.02 | 45.1 ± 1.3   |

<sup>1</sup>Super Prime: 66.3% CP, 11.5% CF, Pesquera Diamante, Peru.

<sup>2</sup>Protein X (defatted *Hermetia illucens* meal): 58% CP, 9% CF, Protix, The Netherlands.

<sup>3</sup>Poultry blood meal: 89% CP, 0.47% CF, SONAC, The Netherlands.

<sup>4</sup>Poultry meal 65: 65% CP, 12% CF, SAVINOR, Portugal.

<sup>5</sup>Wheat bran: 14.8% CP, 4.7% CF, Ribeiro e Sousa, Portugal.

<sup>6</sup>Corn meal: 8.6% CP, 4.3% CF, Casa Lanchinha, Portugal.

<sup>7</sup>PREMIX, Portugal.

<sup>8</sup>DCP: 16.8% P, 20.9% Ca, PREMIX, Portugal.

<sup>9</sup>Sopropêche, France.

<sup>10</sup>SAVINOR, Portugal.

<sup>11</sup>Analysed in duplicate according to standard methods by the accredited laboratory SGS Analytics Germany, Augsburg, Germany; percentages given on as-fed basis; values represent means ± standard deviations.

<sup>12</sup>NFE = 100% – (% CP + % CF + % CFB + % ash + % moisture).

<sup>13</sup>Calculated using the factors 17.15, 23.64, 39.54 MJ/kg for NFE (carbohydrates), CP and CF, respectively (Kleiber, 1961).

## 2.1.2 Experimental system

The experimental system included four recirculating aquaculture systems (RAS) with a total water volume of 460 L, respectively. Each RAS featured a circular rearing tank with a conically shaped bottom (180 L), a settling column for faeces collection with a bottom outlet (10 L) and a biofilter (270 L). The biofilter was partitioned into three equally sized sections, with the first section comprising filter sponge (PPI 10, Schaumstoff-Meister, Straelen, Germany), the aerated centre section holding 30 L of biocarriers (Hel-X HXF12KLL, Christian Stöhr, Marktrodach, Germany) and the last section being a clear water chamber including a circulation pump (Universal 300, EHEIM, Deizisau, Germany). Water flow could be adjusted manually via a valve placed after the circulation pump. Water temperature was controlled by a titanium heater (600 W titanium tube heater, SCHEGO Schemel & Goetz, Offenbach, Germany) and a temperature controller (SCHEGO Schemel & Goetz, Offenbach, Germany). Apart from the aeration in the biofilter, further oxygenation of each RAS was achieved

by an air stone placed in the rearing tanks and lighting was provided by overhead LED lamps. The biofilters of all RAS were matured and synchronized for 6 weeks before the start of the trial by stocking the systems with Nile tilapia and running the RAS in series, i.e., pumping from one RAS to the next and eventually back to the first. One day before introducing the experimental fish, the RAS were emptied, cleaned and refilled with tap water. From this point onward, they were again run separately from each other.

## 2.1.3 Experimental procedure

Mixed-sex Nile tilapia (*Oreochromis niloticus*) originating from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany, were used in the trial. Prior to the trial, fish were reared in a RAS system at 25°C and fed a commercial tilapia diet (Aller Aqua Performa, 2 mm, 45% CP, 20% CF). At the start of the trial, a total number of 342 fish, of which 80 fish were individually weighed and measured (individual body weight: 42.5 ± 11.2 g;

TABLE 2 Elemental composition of fish feed used in the fish feeding trial (dry matter basis).

| Feed | Al     | B     | Ca    | Co    | Cu    | Fe   | K     | Mg   | Mn     | Mo    | Na   | P     | S    | Zn     | N    |
|------|--------|-------|-------|-------|-------|------|-------|------|--------|-------|------|-------|------|--------|------|
|      | mg/kg  | mg/kg | g/kg  | mg/kg | mg/kg | g/kg | g/kg  | g/kg | mg/kg  | mg/kg | g/kg | g/kg  | g/kg | mg/kg  | %    |
| FM   | 243.77 | 4.54  | 31.17 | 0.59  | 17.19 | 0.37 | 11.00 | 2.86 | 68.53  | 1.07  | 4.63 | 22.48 | 6.41 | 100.29 | 6.77 |
| BSFM | 44.45  | 6.35  | 15.89 | 0.11  | 26.23 | 0.21 | 16.27 | 4.55 | 339.75 | 0.96  | 1.29 | 14.09 | 3.85 | 177.16 | 6.96 |
| PM   | 19.65  | 5.69  | 30.46 | 0.45  | 21.78 | 0.20 | 9.83  | 2.18 | 64.22  | 0.73  | 3.94 | 21.22 | 5.55 | 109.00 | 7.38 |
| PBM  | 15.72  | 5.78  | 5.27  | 0.11  | 21.05 | 1.00 | 7.55  | 1.90 | 82.57  | 0.65  | 1.82 | 9.13  | 4.51 | 68.69  | 7.02 |

individual total length:  $132.4 \pm 11.5$  mm;  $n=80$ ), were randomly stocked into the four RAS (82–88 individuals per RAS) such that a starting biomass of 3,500 g per RAS system was achieved, which equated to a rearing density of  $20 \text{ kg/m}^3$ . The fish were hand-fed 80 g of the experimental diets in two equal portions daily for 14 days, except for the first day on which they received half the portion. After this period, the biomass was determined and the RAS were emptied, cleaned, and refilled with tap water again. The fish number in the batch of each dietary treatment was reduced by randomly removing individuals to reach a starting biomass of 3,500 g and batches were again randomly assigned to one of the RAS to be reared for the next 14 days. This procedure was replicated 5 times. Finally, 20 fish per dietary treatment were again weighed and measured individually.

During the trial, a daily water exchange of 7% of the RAS volume (32.2 L) was performed by removing water from the biofilter according to volumetrically defined markings and replenishing it with fresh tap water. Water recirculation in the RAS was set to 180 L/h, which was controlled and if necessary adjusted weekly. Before daily water exchange and feeding, oxygen, pH, temperature (HQ40d, Hach Lange, Berlin, Germany), and electrical conductivity (EC) (pH/Cond 740i, WTW, Weilheim in Oberbayern, Germany) were measured in each rearing tank. During each of the 14-day periods, the faeces were removed from the settling columns daily and the removed sludge water mix was filtered through  $90 \mu\text{m}$  nylon mesh to separate the faeces from the water. The faeces were subsequently collected from the top of the mesh surface and pooled per dietary replicate for each 14-day period and stored at  $-80^\circ\text{C}$ . This approach ensures the full collection of faeces from the system. Before passing the faeces on to the black soldier fly larvae trial, they were homogenized with a blender and freeze-dried until constant weight was achieved.

## 2.2 Black soldier fly larvae feeding trial

The black soldier fly (BSF) strain used in this study was obtained from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany. The population was fed chicken feed (K (11 4) o.K., Agravis Raiffeisen AG, Velten, Germany) and was reared following the method described by Yakti et al. (2022). Prior to the experiment, eggs were manually collected from rearing cages and were allowed to hatch for 24 h on a substrate consisting of 30% dry chicken feed and 70% water. The neonates were let to grow for 6 days and then were separated from the substrate to be used in the BSF larvae feeding trial.

A feeding trial was conducted to assess the growth and mineral composition of black soldier fly larvae (BSFL) provided with the different faeces variants collected from the fish feeding trial. The trial had 5 treatments: The faeces of fish fed with fishmeal (FM), poultry blood meal (PBM), black soldier fly meal (BSFM), poultry by-product meal (PM), and a control. The control treatment consisted of 8% straw, 16% chicken feed (K (11 4) o.K., Agravis Raiffeisen AG, Velten, Germany) and 77% water, and was prepared as the other substrates. The substrates were prepared by adding water to each of the fish faeces variants to obtain a substrate with 23% dry matter content. The faeces collected from each fish replicate was handled separately without mixing it with other faeces batches obtained from the replicates of the same fish treatment. The substrates were mixed with water using a kitchen spatula and were let to soak in room temperature for 6 h, and then were homogenised and put in polyethylene boxes (area of

12 × 17 cm). Each box contained 325 g of the wet mixture and 325 BSFL (6 days old with an average weight of 2.5 mg) were added on the top of the substrate. The composition of the control feed is shown in Table 3. Each treatment had 5 replicates and the larvae were grown in a chamber with 27°C and 50% relative humidity.

The single-larva weight was monitored daily by collecting and weighing 30–50 larvae per box, and the larvae were thereafter returned to the box. The harvest took place on the 10<sup>th</sup> day of the experiment. The PM treatment was harvested on day 7 due a loss of weight between day 6 and 7. In order to avoid the differential drying of substrates before full consumption, which is a known issue in using fish wastes as a BSFL substrate (Liland et al., 2023), the boxes were weighed daily, and water (27°C) was added to the boxes from day 4 to maintain a total weight of 150 g per box and ensure sufficient moisture (~ 50%) in the substrate.

The larvae were harvested and counted manually to calculate the mortality rate and dried at 60°C until no further weight reduction was recorded. The feed conversion ratio (FCR) was calculated on a dry basis by dividing the dry substrate reduction by the dry BSFL weight gain.

## 2.3 Chemical analyses

The feed provided to the fish in the fish trial, the faeces collected ( $n=5$ ), and the larval biomass produced ( $n=5$ ) were analysed for aluminium (Al), boron (B), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), phosphorus (P), sulphur (S), zinc (Zn), and nitrogen (N). All analyses were done as mentioned in Yakti et al. (2022) in two technical replicates. Briefly, for the elements other than N, a microwave digestion (MARS Xpress, CEM, Matthews, North Carolina) was conducted following LUFA methods Vol. III, 10.8.1.2. followed by multi-element analysis using ICP-OES (DIN EN ISO 11885) with an ICP emission spectrometer (iCAP 6,300 Duo MFC, Thermo, Waltham, MA, United States). N concentration was assessed using an elemental analyser (Vario MAX, Elementar Analysensysteme GmbH, Hanau, Germany), following LUFA Bd. III, 4.1.2. The crude protein in the produced BSFL biomass was calculated from the N content using a factor of 4.43 (Smets et al., 2021).

## 2.4 Statistical analyses

The data was analysed using SPSS (IBM Corp, Armonk, NY, USA). The parameters were analysed using one-way ANOVA ( $p=0.05$ ) followed by the consequent Tukey's HSD after confirming data normality and homogeneity of variance. Kruskal-Wallis test was used when the data did not meet the assumption of the parametric test also after applying common data transformations. Spearman's rank correlation was used to evaluate the linear relation between the elements' concentration in the BSFL and the fish faeces (BSFL substrate).

# 3 Results

## 3.1 Fish feeding trial

Rearing condition during the RAS trial were within acceptable limits for tilapia regarding mean oxygen levels (6.7–6.9 mg/L),

TABLE 3 The elemental composition of the control substrate (chicken feed and straw) used in the BSFL feeding trial (dry matter basis).

| Al     | B     | Ca    | Co    | Cu    | Fe   | K     | Mg   | Mn    | Mo    | Na   | P    | S    | Zn    | N    |
|--------|-------|-------|-------|-------|------|-------|------|-------|-------|------|------|------|-------|------|
| mg/kg  | mg/kg | g/kg  | mg/kg | mg/kg | g/kg | g/kg  | g/kg | mg/kg | mg/kg | g/kg | g/kg | g/kg | mg/kg | %    |
| 400.47 | 10.25 | 19.90 | 0.29  | 13.25 | 0.39 | 10.83 | 1.65 | 90.08 | 0.96  | 0.98 | 3.21 | 1.76 | 62.72 | 1.38 |



TABLE 4 Fish growth performance and faeces collection.

|   | BSFM                      | FM                       | PBM                       | PM                       |
|---|---------------------------|--------------------------|---------------------------|--------------------------|
| % Survival <sup>A</sup>                           | 99.8 ± 0.5                | 100.0 ± 0.0              | 100.0 ± 0.0               | 99.7 ± 0.6               |
| Biomass gain (g) <sup>A</sup>                     | 539 <sup>b</sup> ± 63     | 1001 <sup>a</sup> ± 70   | 416 <sup>c</sup> ± 62     | 901 <sup>a</sup> ± 115   |
| Feed conversion ratio (FCR) <sup>3A</sup>         | 2.03 <sup>b</sup> ± 0.22  | 1.08 <sup>c</sup> ± 0.07 | 2.65 <sup>a</sup> ± 0.38  | 1.22 <sup>c</sup> ± 0.15 |
| Feed administered over 14 days (g)                | 1,080                     | 1,080                    | 1,080                     | 1,080                    |
| Faeces DM collected over 14 days (g) <sup>A</sup> | 104.6 <sup>b</sup> ± 10.9 | 97.2 <sup>b</sup> ± 3.7  | 234.0 <sup>a</sup> ± 14.9 | 105.0 <sup>b</sup> ± 9.0 |

Values represent means ± standard deviations; means in rows with different superscript letters are significantly different ( $n = 5$ ,  $p < 0.05$ ). <sup>A</sup> $n = 5$ ; <sup>3</sup>FCR = total feed administered (g as fed) / [final biomass (g) – initial biomass (g)].

temperature (25.5°C), water pH (6.9–7.8), and electrical conductivity (937–1,026  $\mu\text{S}/\text{cm}$ ) in all treatments and results are compiled in Table S1. The fish biomass gain differed based on the feed provided (ANOVA followed by Tukey's test on the log10 transformed data,  $p < 0.001$ ,  $F = 51.56$ ), and the values ranged from 415 g in the fish that received poultry blood meal (PBM) to 1 kg for the fish that were provided with fish meal (FM) (Table 4). No differences were observed between the growth of fish that received poultry meal (PM) and FM, but the growth decreased significantly in the black soldier fly meal (BSFM)-fed fish, and PBM-fed fish. Results for initial and final biomass, body weight and condition factor as well as specific growth rate can be found in Table S2.

The feed conversion ratio (FCR) of the fish was calculated and significantly differed among the treatments (ANOVA followed by Tukey's test on the log10 transformed data,  $p < 0.001$ ,  $F = 51.54$ ). Besides having the lowest biomass gain and producing the highest amount of faeces, the fish provided with the PBM had the highest FCR followed by the BSFM-fed fish. The best growing fish-treatments (FM and PM) had the lowest FCR values.

The amount of faeces collected also differed among the treatments (ANOVA followed by Tukey's test on the log10 transformed data,  $p < 0.001$ ,  $F = 121$ ). The PBM-fed fish produced the highest amount of faeces (234 g dry faeces), while the rest of treatments did not differ among each other and produced approximately 100 g dry faeces (Table 4).

In addition to the amounts of fish faeces, the chemical composition of the faeces varied significantly based on the feed provided (Table 5). The faeces collected from the FM-fed fish had the highest concentration of Al, B, Ca, Co, Mg, Mo, Na, and P. The faeces collected from the BSF-fed fish contained the highest concentration of Cu, Mn, and Zn. High Zn was also observed in the PM and FM faeces. The faeces collected from the PBM-fed fish contained the lowest concentration of Al, B, Ca, Co, Cu, K, Mn, Mo, Na, P, and Zn, but was the richest in Fe, S and N.

### 3.2 Black soldier fly larvae feeding trial

The performance of black soldier fly larvae (BSFL) was compared when cultivated on the different fish faeces and the control substrate. The peak weight of the BSFL was reached on day 6 for the PM treatment so the larvae had to be harvested on the seventh. The other treatments, including the control, were harvested on day 10. The single-larva weight at the end of the experiment was the highest in the larvae provided with the control diet and 0.088 g (SD = 0.003) ( $p < 0.001$ ,  $F = 272$ ). No differences in the single-larva weight were found between the different faeces' variants, and they reached a weight

from 0.0228 to 0.0219 with SD values ranging from 0.0024 to 0.0059. The total biomass gains of BSFL did not differ among the faeces treatments, and the highest growth was observed in the control treatment (Figure 1).

The harvested BSFL were manually counted to calculate the mortality rate and no differences were found (Figure 2).

The feed conversion ratio (FCR) was calculated and differed among the treatments. The control BSFL had the lowest FCR, and the values did not significantly differ from the BSFL that received the PBM faeces. Among the fish faeces treatments, differences were only observed between the PBM and the PM BSFL (Figure 3).

The elemental composition of the BSFL differed significantly based on the fish faeces received (Table 6). Generally, the larvae that consumed fish faeces had lower Al, B, and Co concentration than the faeces (initial substrate). Higher concentrations of Ca, K, Mg, Mn, Na, S, and Zn were observed. The values of Cu, Fe, Mo, P were higher or lower based on the treatment (Table 6). Providing the BSFL with the control substrate led to higher Al and B, and lower Zn, Cu, and P in some fish faeces variants. The concentration of all analysed elements, namely Al, B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, and Zn differed based on the fish faeces provided. Providing the BSFL with FM faeces led to the highest values of Al, B, Ca, Co, Cu, Mo, and P while the values of other elements were closer to other treatments. The BSF-meal derived fish faeces led to the highest Mn and Zn values in the larvae. The BSFL given the PBM faeces accumulated the highest amounts of S and had the highest crude protein values.

A very strong significant correlation was found between the concentrations of Al, P, S, Zn, and N in the BSFL and their concentrations in the fish faeces they fed on (Spearman's  $\rho > 0.75$ ,  $p < 0.001$ ). Significant correlations (Spearman's  $\rho > 0.5$ ,  $p < 0.01$ ) were also found in the case of B, Ca, Cu, Fe, Mg, and Mo. No significant correlations were found in K, Mn, and Na (Figure 4).

## 4 Discussion

The rapid expansion of aquaculture is raising sustainability concerns due to two primary factors: the dependence on marine fish meal obtained from wild fish stocks for the production of fish feed (Stankus, 2021), and the environmental repercussions primarily associated with the generation of waste (Dauda et al., 2019). The high dependence on marine FM has led to mounting pressure on wild fish populations, resulting in the rapid depletion of wild fish stocks (Stankus, 2021). Research has been carried out to explore alternative protein sources that offer comparable nutritional value to marine fish meal (Daniel, 2018). These protein sources, among others, include poultry by-product meal

TABLE 5 The elemental composition of the faeces collected from the fish provided with different feed.

| Treatment | Al<br>mg/kg | B<br>mg/kg          | Ca<br>g/kg         | Co<br>mg/kg        | Cu<br>mg/kg       | Fe<br>g/kg          | K<br>g/kg         | Mg<br>g/kg        | Mn<br>mg/kg          | Mo<br>mg/kg       | Na<br>g/kg        | P<br>g/kg           | S<br>g/kg         | Zn<br>mg/kg         | N<br>%             |  |
|-----------|-------------|---------------------|--------------------|--------------------|-------------------|---------------------|-------------------|-------------------|----------------------|-------------------|-------------------|---------------------|-------------------|---------------------|--------------------|--|
| FM        | Mean        | 598.33 <sup>b</sup> | 9.42 <sup>b</sup>  | 74.69 <sup>b</sup> | 1.43 <sup>b</sup> | 57.69 <sup>ab</sup> | 0.83 <sup>c</sup> | 2.58 <sup>c</sup> | 192.86 <sup>ab</sup> | 2.02 <sup>b</sup> | 1.17 <sup>c</sup> | 35.68 <sup>a</sup>  | 3.54 <sup>a</sup> | 323.88 <sup>b</sup> | 2.55 <sup>c</sup>  |  |
|           | SD          | 72.24               | 0.79               | 2.90               | 0.31              | 3.78                | 0.07              | 0.12              | 15.03                | 0.07              | 0.09              | 1.28                | 0.06              | 14.21               | 0.09               |  |
| BSF       | Mean        | 133.23 <sup>b</sup> | 8.55 <sup>ab</sup> | 29.71 <sup>a</sup> | 0.52 <sup>a</sup> | 66.77 <sup>b</sup>  | 0.83 <sup>c</sup> | 2.57 <sup>c</sup> | 581.27 <sup>a</sup>  | 1.82 <sup>a</sup> | 0.63 <sup>b</sup> | 15.08 <sup>bc</sup> | 3.44 <sup>a</sup> | 498.98 <sup>a</sup> | 3.98 <sup>ab</sup> |  |
|           | SD          | 8.41                | 0.99               | 1.43               | 0.09              | 0.59                | 0.05              | 0.13              | 67.24                | 0.08              | 0.03              | 0.69                | 0.13              | 8.46                | 0.051              |  |
| PM        | Mean        | 55.46 <sup>ab</sup> | 8.14 <sup>ab</sup> | 62.2 <sup>d</sup>  | 1.05 <sup>b</sup> | 65.37 <sup>b</sup>  | 0.63 <sup>b</sup> | 1.82 <sup>b</sup> | 174.38 <sup>bc</sup> | 1.43 <sup>d</sup> | 1.05 <sup>c</sup> | 30.49 <sup>ab</sup> | 3.59 <sup>a</sup> | 360.92 <sup>d</sup> | 2.9 <sup>bc</sup>  |  |
|           | SD          | 6.76                | 1.50               | 7.39               | 0.13              | 8.91                | 0.07              | 0.06              | 21.80                | 0.07              | 0.09              | 3.13                | 0.07              | 12.41               | 0.084              |  |
| PBM       | Mean        | 26.52 <sup>a</sup>  | 7.22 <sup>a</sup>  | 11.02 <sup>c</sup> | 0.38 <sup>a</sup> | 34.88 <sup>a</sup>  | 0.21 <sup>a</sup> | 1.25 <sup>a</sup> | 87.48 <sup>c</sup>   | 0.65 <sup>c</sup> | 0.33 <sup>a</sup> | 8.59 <sup>b</sup>   | 6.74 <sup>b</sup> | 122.66 <sup>c</sup> | 9.94 <sup>a</sup>  |  |
|           | SD          | 4.73                | 0.71               | 0.86               | 0.04              | 3.05                | 0.59              | 0.14              | 13.22                | 0.02              | 0.06              | 0.41                | 0.20              | 7.38                | 0.72               |  |
| F         |             |                     |                    |                    |                   |                     | 122.99            | 149.73            |                      | 446.64            | 148.90            |                     | 812.56            | 1002.85             |                    |  |
| H         |             |                     |                    | 16.14              | 14.72             | 16.28               |                   |                   | 16.71                |                   |                   | 17.86               |                   |                     | 17.86              |  |

Shown are the means and standard deviations (SD) of the elements quantified on a dry matter basis. The different groups are expressed with the different letters above the mean values. The F-values are shown for one-way ANOVA, and the H- (test statistics) values are shown for Kruskal-Wallis test.

(Galkanda-Arachchige et al., 2020), insect meal (Oteri et al., 2021), and poultry blood meal (Luthada-Raswiswi et al., 2021). Such protein sources do not only serve as alternatives to FM but also adhere to the principles of the circular bioeconomy (Stegmann et al., 2020) in that they represent wastes streams or potential biowaste converting biomass (in case of insect meal) that can be upcycled into higher value protein sources for human consumption via aquaculture. Also, they are not subject to food-feed conflict as other feed raw materials such as FM from capture fisheries or many plant protein sources (Colombo and Turchini, 2021; Colombo et al., 2022). Accordingly, they can play an important role in achieving greater circularity in aquafeed production.

### 4.1 The fish feeding trial

The use of black soldier fly meal (BSFM) as a fish feed component has been widely investigated in different fish species (Alfiko et al., 2022). In the case of Nile tilapia, some studies have suggested that growth and feed intake do not differ between fish fed on commercial fish meal (FM) or black soldier fly meal (BSFM) (Tippayadara et al., 2021). Limbu et al. (2022) have demonstrated that replacing 75% of the FM with BSFM in an initial diet with 42% FM lead to an improved fish biomass gain and growth rate in Nile tilapia fry, and pointed to improved economic returns in the production system. In our study, replacing FM with BSFM led to an almost 50% reduction in biomass gain (Table 4). Both feeds, despite having minimal differences in nitrogen content, could have significantly differed in actual protein content since BSFM can contain certain amounts of mineral N or N integrated into the chitin skeleton, leading to a lower N-to-protein transformation factor compared to other protein sources (Smets et al., 2021). This may have contributed to the reduced fish growth. A meta-analysis on the inclusion level of insect meals in aquafeeds suggests maximum inclusion levels of 25–30% for unimpaired growth performance (Liland et al., 2021), and another meta-analysis suggests a maximum 29% BSFM inclusion in aquafeeds (Hua, 2021). In line with the results of the present study, other authors also found reduced growth performance above certain levels of FM replacement with BSFM for tilapia (Taufek et al., 2021). Considering that the BSFM was the sole dietary protein source in the BSF diet of the present study and BSFM inclusion level (61.6%) was higher than in the above studies (8–30%), it appears reasonable to assume that excessively high dietary BSFM inclusion, particularly if not complemented by other protein ingredients, is not conducive to maximum growth performance in tilapia. One reason for this could be chitin impairing protein digestibility, especially at high BSFM inclusion levels, as suggested by some authors (Shiau and Yu, 1999; Kroeckel et al., 2012; Rana et al., 2015).

The second FM alternative used in the experiment was poultry by-product meal (PM), which is generally known to be a valuable protein source and alternative to fish meal in aquafeeds. PM is made from the processed rendered parts of poultry carcasses, which include feet, heads and parts not typically consumed by human. In a meta-analysis, Galkanda-Arachchige et al. (2020) showed that many freshwater fish species readily accept above 50% FM replacement with PM and 100% replacement can be possible without adverse effects. Although some studies have shown that PM can lead to lower protein use efficiency and digestibility in Nile tilapia (El-Sayed, 1998; Palupi et al., 2020). Hernández et al. (2010), and Yones and Metwalli (2015) observed similar growth performance and feed utilisation even at

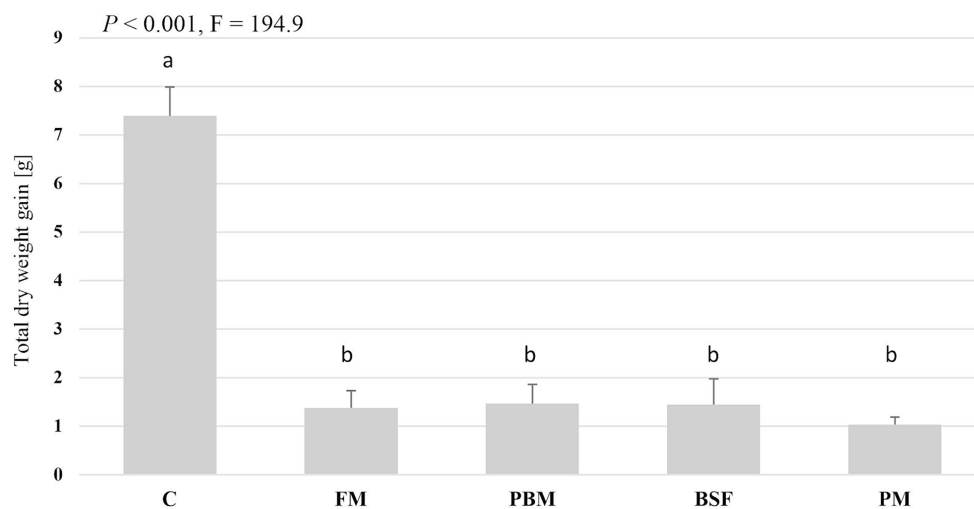


FIGURE 1

The dry biomass gain of black soldier fly larvae (BSFL) fed with different fish faeces. BSFL were grown on faeces collected from fish that received fishmeal (FM), blood meal (PBM), black soldier fly meal (BSF), poultry by-product meal (PBM), or a control substrate (chicken feed and straw), and the dry biomass gain was calculated. The analysis of variance ANOVA ( $n = 5$ ,  $p < 0.05$ ) revealed only a significant difference between the control treatment and the faeces treatments.

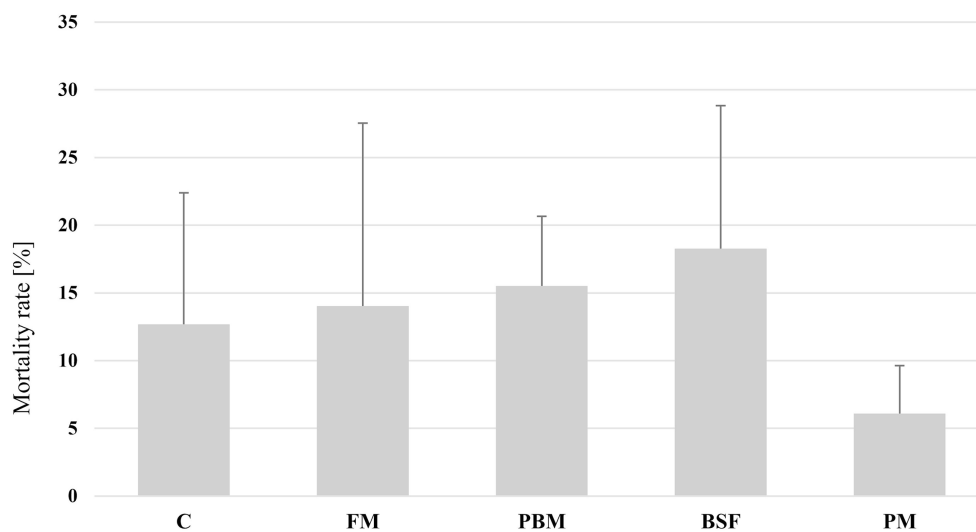


FIGURE 2

The mortality rate of black soldier fly larvae (BSFL) grown on different fish faeces. BSFL were grown on faeces collected from fish that received fishmeal (FM), blood meal (PBM), black soldier fly meal (BSF), poultry by-product meal (PBM), or a control substrate (chicken feed and straw). The larvae were harvested, and the survival was assessed. The analysis of variance ANOVA ( $n = 5$ ,  $p < 0.05$ ) revealed no difference between the treatments.

100% FM replacement with PM, which is consistent with the results of this study as the biomass gain and feed conversion ratio (FCR) were not compensated when FM was substituted with PM (Table 4). FCR is a critical parameter in evaluating livestock performance as it measures the efficiency with which animals convert feed into body mass. The partial or full replacement of fishmeal with a more sustainable or lower cost component without influencing the FCR can boost the profitability and sustainability in aquaculture systems.

PBM as the third protein source, although resulting in a diet which compared mostly favorably in terms of its essential amino acid profile to the other diets (Shaw et al., 2022a) led to the lowest biomass gain and the highest faeces production, and consequently the highest FCR (Table 4). PBM has been shown to negatively affect Nile tilapia growth

when replacing FM (Otubusin, 1987; El-Sayed, 1998; Kirimi et al., 2016) and in the case of the present study, the comparably low Ca and P content of the PBM diet, which were both slightly below the requirements for Nile tilapia (Mjoun et al., 2010), supposedly played a role in suppressing the growth performance of the fish. Subsequently, mineral Ca and P supplementation or complementing PBM with other P-/Ca-rich protein sources, i.e., terrestrial animal by-products such as PM, would likely improve the suitability of PBM at high inclusion levels in diets for Nile tilapia. Despite the reduced growth and feed conversion performance achieved with the BSF and the PBM diet in this and prior studies (Shaw et al., 2022b; a), the nutritional value of BSFM and PBM should not be discounted, since in combination with complementary raw materials such as PM or catfish by-product meal they have the



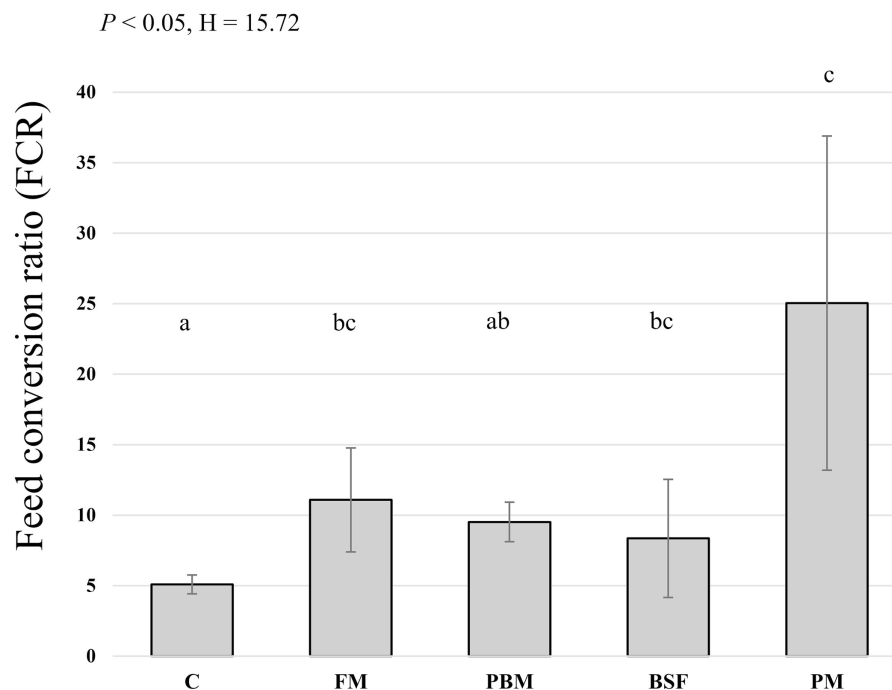


FIGURE 3

The feed conversion ratio (FCR) of black soldier fly larvae (BSFL) grown on different fish faeces. BSFL were grown on faeces collected from fish that received fishmeal (FM), blood meal (PBM), black soldier fly meal (BSF), poultry by-product meal (PBM), or a control substrate (chicken feed and straw), and the feed conversion ratio (FCR) was calculated. Kruskal-Wallis test ( $n = 5$ ,  $p < 0.05$ ) revealed significant differences between the treatments and are represented by the different letters above the columns.

potential to rival the growth performance achievable with commercially available feed (Shaw et al., 2023). To this effect, modern feed formulation in aquaculture should not be a search for FM or fish oil substitutes but rather have the goal of identifying combinations of complementary raw materials which together fulfil the nutritional requirements of the species in question as well as achieving further relevant objectives (waste reduction, low contaminant levels, cost, etc.).

Besides the above-described differences in fish growth, the experimental feeds resulted in differences in the elemental composition of the respective faeces (Table 5). Since fish excrete excess nutrients in dissolved forms via brachial and urinary excretion and in solid form via faeces, it is to be expected that dietary differences in elemental composition are at least partly reflected in the elemental composition of the respective faeces. This was apparent in the case of, e.g., Fe in this study, which showed the highest level in the PBM feed and subsequently in the PBM-derived faeces. Similar relations between elemental content in the fish feed and the resulting content in the faeces were for the most part found for Ca, K, Mg, Na, P, Zn, but not for S. In the case of N, the highest content as well as total amount was found in the PBM-derived faeces, while the opposite was observed in the faeces generated from the FM treatment. Considering that the PBM diet enabled the least favorable growth performance, this could indicate that the N in the PBM diet was not efficiently utilized by the fish. It can be assumed that growth inhibition was due to other limiting factors such as the low Ca and P content or lower digestibility of protein in the PBM compared to the other protein sources.

Overall, the results of the present fish rearing trial corroborate findings of a previous study in which the same diets were fed to Nile tilapia in a different experimental RAS setup (Shaw et al.,

2022b), with similar differences found between the dietary treatments regarding fish growth and feed conversion performance, as well as faeces amounts and respective elemental compositions.

## 4.2 Black soldier fly larvae feeding trial

With the growing focus on circularity in food production systems, the recognition of fish faeces as a waste resource is increasing, necessitating strategic management practices. Generally, the assessment of aquaculture sludge as a substrate for insect production is limited and has been rarely discussed in literature (Schmitt et al., 2019; Liland et al., 2023). This could be due to the legal restrictions of using fish wastes as a substrate in production, the relatively poor growth of BSFL on fish faeces in comparison to many other organic waste streams, or the associated risks of contaminants. The accumulation of heavy metals in BSFL is considered to be a major issue when growing BSFL on waste streams and manure (Diener et al., 2015; Proc et al., 2020), and fish faeces, in particular, can feature high amounts of heavy metals (Lalander et al., 2013; Schmitt et al., 2019) and other unwanted elements which originate from the fish feed and are unabsorbed by the fish. The modulation of fish faeces composition by strategically choosing raw materials low in these contaminants and manipulating fish feed formulations accordingly, without compensating fish growth, can be a strategy to generate fish faeces that may be more suitable to be recycled by BSFL with reduced associated risks. To the best of our knowledge, the assessment of BSFL performance and composition on faeces stemming from fish fed different diets has not been done in published literature.

TABLE 6 The elemental composition BSFL provided with different fish faeces or a control (C).

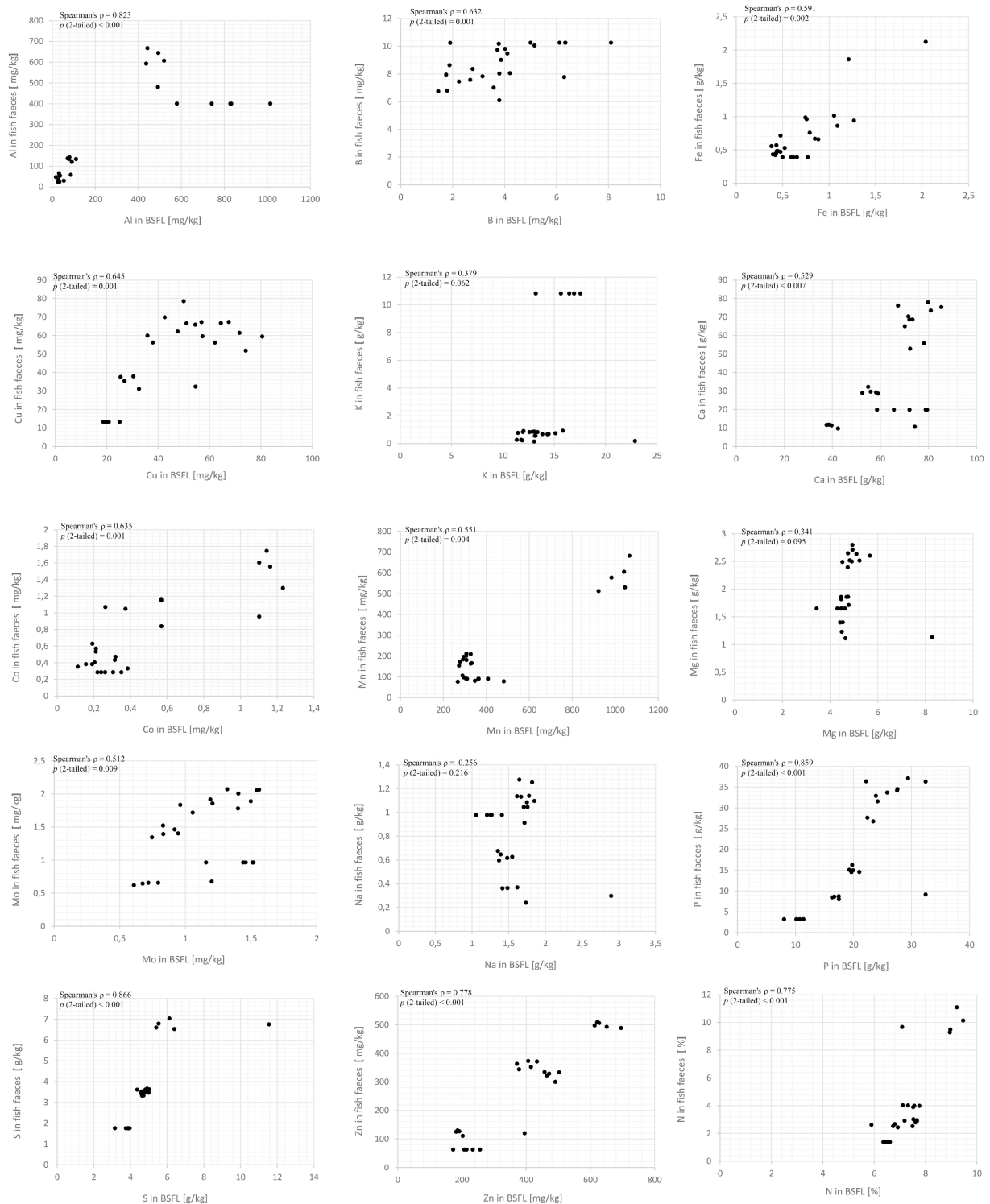
| Treatment | Al [mg/kg] | B [mg/kg]            | Ca [g/kg]          | Co [mg/kg]          | Cu [mg/kg]        | Fe [g/kg]           | K [g/kg]          | Mg [g/kg]           | Mn [mg/kg]        | Mo [mg/kg]           | Na [g/kg]          | P [g/kg]           | S [g/kg]            | Zn [mg/kg]        | Protein (N x 4.43)  |                    |
|-----------|------------|----------------------|--------------------|---------------------|-------------------|---------------------|-------------------|---------------------|-------------------|----------------------|--------------------|--------------------|---------------------|-------------------|---------------------|--------------------|
| FM        | Mean       | 476.97 <sup>bc</sup> | 4.24 <sup>b</sup>  | 77.02 <sup>b</sup>  | 1.15 <sup>b</sup> | 69.13 <sup>c</sup>  | 0.80 <sup>b</sup> | 14.09 <sup>bc</sup> | 5.11 <sup>b</sup> | 295.67 <sup>b</sup>  | 1.46 <sup>c</sup>  | 1.71 <sup>b</sup>  | 27.83 <sup>c</sup>  | 4.85 <sup>b</sup> | 477.33 <sup>b</sup> | 30 <sup>b</sup>    |
|           | SD         | 35.68                | 0.54               | 7.35                | 0.05              | 9.38                | 0.06              | 1.29                | 0.35              | 12.10                | 0.10               | 0.09               | 3.74                | 0.17              | 18.96               | 2.54               |
| BSF       | Mean       | 86.40 <sup>c</sup>   | 2.98 <sup>bc</sup> | 56.12 <sup>ab</sup> | 0.22 <sup>a</sup> | 58.85 <sup>c</sup>  | 0.45 <sup>a</sup> | 12.18 <sup>a</sup>  | 4.81 <sup>b</sup> | 1011.8 <sup>a</sup>  | 1.16 <sup>b</sup>  | 1.43 <sup>a</sup>  | 19.93 <sup>b</sup>  | 4.59 <sup>b</sup> | 641.50 <sup>c</sup> | 33.03 <sup>b</sup> |
|           | SD         | 15.24                | 0.81               | 2.62                | 0.05              | 6.87                | 0.05              | 0.56                | 0.22              | 58.79                | 0.17               | 0.08               | 0.65                | 0.14              | 33.21               | 1.09               |
| PM        | Mean       | 41.08 <sup>a</sup>   | 2.15 <sup>c</sup>  | 73.22 <sup>ab</sup> | 0.47 <sup>b</sup> | 42.77 <sup>b</sup>  | 0.43 <sup>a</sup> | 13.79 <sup>bc</sup> | 4.63 <sup>a</sup> | 313.83 <sup>b</sup>  | 0.85 <sup>a</sup>  | 1.75 <sup>b</sup>  | 23.94 <sup>bc</sup> | 4.84 <sup>b</sup> | 400.81 <sup>b</sup> | 33.36 <sup>b</sup> |
|           | SD         | 26.60                | 0.97               | 2.97                | 0.14              | 6.05                | 0.03              | 0.63                | 0.16              | 24.63                | 0.08               | 0.07               | 1.25                | 0.06              | 26.04               | 0.90               |
| PBM       | Mean       | 35.15 <sup>a</sup>   | 3.81 <sup>bc</sup> | 46.49 <sup>a</sup>  | 0.23 <sup>a</sup> | 33.93 <sup>ab</sup> | 1.33 <sup>b</sup> | 14.18 <sup>ab</sup> | 5.27 <sup>a</sup> | 336.02 <sup>b</sup>  | 0.78 <sup>a</sup>  | 1.83 <sup>ab</sup> | 20.08 <sup>ab</sup> | 7.00 <sup>b</sup> | 231.64 <sup>a</sup> | 38.7 <sup>c</sup>  |
|           | SD         | 10.89                | 1.51               | 15.65               | 0.11              | 11.86               | 0.41              | 4.89                | 1.69              | 86.40                | 0.23               | 0.61               | 6.93                | 2.58              | 91.88               | 4.19               |
| C         | Mean       | 799.32 <sup>b</sup>  | 6.38 <sup>a</sup>  | 70.94 <sup>ab</sup> | 0.27 <sup>a</sup> | 20.81 <sup>a</sup>  | 0.62 <sup>a</sup> | 15.96 <sup>c</sup>  | 4.26 <sup>a</sup> | 350.51 <sup>ab</sup> | 1.42 <sup>bc</sup> | 1.23 <sup>a</sup>  | 10.11 <sup>a</sup>  | 3.72 <sup>a</sup> | 216.80 <sup>a</sup> | 28.6 <sup>a</sup>  |
|           | SD         | 157.88               | 1.11               | 8.90                | 0.05              | 2.47                | 0.10              | 1.69                | 0.48              | 42.57                | 0.15               | 0.13               | 1.26                | 0.33              | 31.31               | 0.46               |
| F         |            |                      | 11.75              |                     | 29.17             |                     |                   |                     |                   | 19.66                |                    |                    |                     |                   | 68.11               | 14.44              |
| H         |            | 21.2                 |                    | 13.83               | 17.25             | 21.4                | 13.23             | 12.88               | 14.95             |                      | 17.66              | 17.91              | 21.15               |                   |                     |                    |

Shown are the means and standard deviations (SD) of the elements quantified in the larvae. The different groups are expressed with the different letters above the mean values. The F-values are shown for one-way ANOVA, and the H- (test statistics) values are shown for Kruskal-Wallis test.

As anticipated, the growth of BSFL on all faeces types was lower compared to the control treatment (a mixture of chicken feed and straw) (Figure 1), which was also reflected in the FCR values (Figure 3). Nevertheless, the fish faeces treatments did differ among each other in terms of insect biomass gain. The growth of the BSFL in this study, however, was considerably lower than that obtained by Liland et al. (2023) and Schmitt et al. (2019) who grew BSFL on aquaculture sludge obtained from commercial RAS facilities. In contrast to small and highly controlled experimental systems such as used in the present study, aquaculture sludge from commercial RAS can contain noteworthy amounts of unconsumed fish feed (Madariaga and Marín, 2017; Campanati et al., 2022) and perhaps even parts of fish carcasses, which may be factors explaining the difference in growth observed between the above mentioned studies and the presented trial which relied on pure faeces from an experimental RAS. In this sense, also the faeces collection method (e.g., sedimentation units as used in this trial vs. drum filters commonly used in commercial RAS) and residence time of sludge in the system water may influence not only the quantity but also the nutritional quality of the collected sludge. The FCR values observed for the fish faeces-fed BSFL (5.3 to 25.4) were comparable to the values obtained for pig manure (Newton et al., 2005), chicken manure (Sheppard et al., 1994) and are lower than those obtained from municipal organic wastes (Diener et al., 2011). The PBM-derived faeces contained the highest N value but, despite not differing from the control treatment in the FCR value (Figure 3), did not yield in a higher BSFL growth. This is likely due to a limiting nutrient other than protein, or the lower digestibility of proteins in PBM. However, the use of PBM-derived faeces in combination with high-carbohydrate low-protein components could potentially allow better utilisation of the N from the PBM-derived faeces.

The survival of BSFL did not differ among the treatments. It can be assumed that variance in the mineral composition and heavy metal content of the substrates (in the ranges observed) have no influence on mortality. BSFL are known to tolerate and survive on substrates with high concentrations of heavy metals (Cai et al., 2018; Hu et al., 2023; Siddiqui et al., 2023), but this can lead to the accumulation of certain heavy metals as shown for Cd, Pb and Zn from chicken feed and contaminated substrates (Diener et al., 2015; Van der Fels-Klerx et al., 2016). Generally, BSFL can accumulate higher concentrations of S, Ca, Mg, Mn, Zn, Fe and K than present in the rearing substrate when provided with a balanced diet such as chicken feed (Yakti et al., 2022). Such enrichment improves the nutritional value of larvae intended for the use as raw material for animal feeds (Shumo et al., 2019). In a study in which BSFL were given a substrate supplemented with very high concentrations of As, Cd, Cr, Hg, Ni, Pb, a reduction of growth was observed but only Cd and Pb accumulated (Purschke et al., 2017). Cai et al. (2018) have pointed at a link between the concentration of Zn and Cu in a municipal sewage sludge substrate and larval mortality. The concentrations were, however, higher than those found in the sampled faeces in this study.

In the present study, the concentration of analysed elements in the BSFL differed among treatments (Table 6) and the relation was assessed with a correlation analysis (Figure 4). Significant correlations in the case of Al, B, Fe, Cu, Ca, Co, Mg, Mo, P, S, Zn, and N were detected. Relations between initial concentration of elements in the feeding substrates and the produced BSFL biomass have been already observed in published studies that included gradients of wastes into BSFL substrate. Liland et al. (2017), for example, cultivated BSFL on



**FIGURE 4** The relation between the concentration of elements in the insects rearing substrate (Fish faeces) and the concentration of the elements in the produced BSFL biomass. Shown are the plotted values, the spearman's correlation coefficient, and the p-values.

seaweed-enriched substrates and found an accumulation of Cd, Pb, Hg, and As in the BSFL. The concentrations of these elements in the larvae increased proportionally with increasing levels of seaweed inclusion in the substrate. In a similar approach, [Liland et al. \(2023\)](#) mixed chicken feed with different ratios of aquaculture sludge and

showed that the content of individual minerals and metals was higher in the sludge-fed BSFL compared to those fed with chicken feed. Elements such as Fe, Zn, and Se, which were found in higher amounts in the aquaculture wastes, increased in the BSFL due the increased incorporation of fish faeces in the BSFL substrate.

## 5 Outlook

Circular production systems are gaining interest as they promote resource efficiency, waste reduction, economic benefits, and resilience in supply chains. The ability of insects to process different biowaste streams into higher value insect biomass means they could play a key role in closing nutrient cycles and linking food and agricultural production systems. Given that insects are farmed animals, their rearing should comply with legal frameworks like those established by the European Union (Commission Regulation (EU) 2017/893), and their feeding substrate should meet specific requirements. Aside from the legal issues with using aquaculture sludge as a rearing substrate, the maximum allowed levels of heavy metals and other contaminants must not be exceeded in insects destined for feed production. This study shows that ingredient choice in fish feed formulation cannot only significantly influence fish growth performance but also the elemental composition of the, respectively, produced solid waste streams, i.e., the fish faeces, and these differences in turn can be reflected in the elemental composition of the produced larvae. Along the lines of formulating more sustainable and circular aquaculture feeds and potentially upcycling the solid wastes excreted by the fish via insect larvae production, future research should focus on identifying combinations of complementary raw materials generated within the circular bioeconomy which enable good fish growth and health as well as result in solid wastes low in contaminants such as heavy metals. Considering the low growth performance of larvae reared on the fish faeces in this study, future research could identify complementary biowaste streams that can be complemented by the different elemental compositions and quantity of the collected faeces. Additionally, further studies should focus on altering the accumulation of contaminants such as Cd, Pb, Hg, and As which were not investigated in the current study.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#), further inquiries can be directed to the corresponding authors.

## Ethics statement

The animal study was approved by the Landesamt für Gesundheit und Soziales- Berlin, Germany. The study was conducted in accordance with the local legislation and institutional requirements.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2024.1298885/full#supplementary-material>

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