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Utility of by-products of black soldier fly larvae (*Hermetia illucens*) production as feed ingredients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

Projected growth in insect production as alternative feedstuffs will yield novel by-products that are potentially valuable for aquafeed applications. We analyzed the nutrient composition of three by-products occurring from black soldier fly larvae production (exuvia, cocoon, imago) and the bioavailability of key nutrients for Pacific Whiteleg shrimp (*Litopenaeus vannamei*). Protein accounted for 317 g kg^{-1} in exuviae, 433 g kg^{-1} in cocoons, and up to 521 g kg^{-1} in adult flies (imagines). Considerable amounts of essential amino acids were detected in imago meal, which significantly matched the ideal dietary amino acid composition for penaeid shrimp ($r^2 = 0.66$, $p = 0.0076$). Exuviae and cocoons contained moderate amounts of lipids (64–140 g kg^{-1}), while imagines comprised 356 g kg^{-1} total lipid. Saturated fatty acids predominated in all insect materials (47%–83% of total fatty acids). Chitin concentration was highest in cocoons and exuviae (154 and 139 g kg^{-1}) and low in imagines (51 g kg^{-1}). A feeding trial with shrimp, *L vannamei*, revealed apparent digestibility coefficients of 20%–59% for protein, 24%–54% for energy, 25%–49% for carbon, and 27%–68% for copper. Defatting of imago meal increased the digestibility of protein, energy, and carbon by

77%, 64%, and 61%, respectively. Defatted imago meal can serve as a protein supplement for shrimp diets.

KEYWORDS
alternative feedstuffs, apparent digestibility coefficients, circular economy, insect by-products, nutrients

1 | INTRODUCTION

The global human population is predicted to reach around 9.7 billion by 2050 (United Nations, 2022). One of the major humanitarian challenges is to secure food availability without exceeding planetary boundaries (Campbell et al., 2017). So far, it is estimated that the global food production sector is responsible for 25% of greenhouse gas emissions (Mrówczyńska-Kamińska et al., 2021), a situation requiring drastic change. Reduction of food waste and circular production patterns are required and frame central goals in policy (European Commission, 2020; United Nations, 2021). Within this context, insects may provide a suitable source material for converting organic waste streams into high value protein and lipid sources. However, as a result of previous disease outbreaks (e.g., transmissible spongiform encephalopathies), hygienic and health concerns exist that have to be addressed regarding the use of animal waste streams as feed ingredients (European Commission, 2001). Ruminant proteins, meat-and-bone meal, catering waste, and manure are prohibited as substrates for insects that are to be used as food/feed in the European Union (European commission, 2009a, 2009b). Yet, recent changes in EU legislation have authorized insects for use in aquafeed (European Commission, 2008), poultry, and pig feed (European Commission, 2021), unlocking further applications for this novel live-stock.

The larvae of the black soldier fly (BSF) (*Hermetia illucens*, Diptera: Stratiomyidae) thrives on numerous organic waste streams (Gold et al., 2018; Jucker et al., 2020; Spranghers et al., 2017) and is an ideal candidate for establishing a circular bio-economy (Liu et al., 2022). In Europe, the technology for industrial insect production is rapidly advancing (Derrien & Boccuni, 2018). In 2018, total insect production was estimated to be 2000 metric tons and is expected to reach 1.2 million metric tons by 2025 (Wunsch, 2020). The total annual monetary turnover of insect feed operators is projected to exceed 2 billion euros by 2023 (IPIFF, 2021), demonstrating the growing market of this industry. Most insect producers in Europe are farming BSFs (Derrien & Boccuni, 2018). Late larvae stages are the main product, and the nutritious larva meal is used as feed for poultry, livestock, and aquaculture (Henry et al., 2015; Makkar et al., 2014; Spranghers et al., 2017). For the production of larvae, all life stages of this insect, from egg to the adult fly (imago), are cultivated (Tomberlin et al., 2002), which, in turn, generates certain by-products.

After hatching, *H. illucens* passes through five larval stages before developing into the pupae (Soetemans et al., 2020). Between each stage, larvae shed their exuviae in order to grow (Hahn et al., 2022). In the pupa stage, the insect metamorphizes into the adult fly (imago) (Tomberlin & Sheppard, 2002). The empty cocoons of the pupae provide another residue of BSF larvae production. Sexual reproduction of *H. illucens* takes place in the imago stage and after a few days of laying eggs, the adult fly dies (Tomberlin & Sheppard, 2002). These three BSF larvae production residues (exuviae, cocoons, and imagines) have been investigated as chitin sources (Hahn et al., 2022; Soetemans et al., 2020), but there is a dearth of research on their potential as sources of biomolecules and nutrients for aquaculture, in particular shrimp species.

The current study presents a detailed characterization of the chemical composition of these insect residues and discusses their relevance for shrimp nutrition. A controlled digestibility trial with Pacific Whiteleg shrimp (*Litopenaeus vannamei*) demonstrates the bioavailability of key nutrients and provides baseline information to investigate the potential of these materials as ingredients for aquafeed production.

2 | MATERIALS AND METHODS

2.1 | By-products of BSF larvae production

Exuviae, cocoons, and imago of BSFs (Figure 1) were obtained from a local insect farm (Farmcycle GmbH, Bremen, Germany). After collection at the production site, the raw materials were transported to the facilities of the Alfred Wegener Institute in Bremerhaven, Germany, and stored at −20°C. The insect materials were oven dried at 60°C for 48 h in glass dishes. After drying, exuviae, cocoons, and imagines were ground to a fine powder with a knife mill (GM 200, Retsch, Germany) in intervals of 30 s with 30 s pauses in between to avoid excess heat development. This process was repeated until all particles of the ground material passed through a 500-µm sieve. The final homogenous meals of the insect by-products were stored at 4°C in air tight containers until further use.

Defatting of the imago meal was done with 99.8% ethanol puriss (Sigma-Aldrich, Germany) as described by L'hocine et al. (2006) and Zhao et al. (2016). Fat extraction was performed at room temperature in 1-L glass bottles at a solvent to meal ratio of 3:1 (v/w) and under continuous rotation of the horizontally placed bottles on a tilt/roller mixer (RS-TR05, Phoenix Instrument, Germany). After 60 min of extraction and another settling period of 30 min in the upright bottles, the lipid-containing supernatant was carefully removed using a glass pipette and discarded. The remaining meal was spread onto glass dishes, left to vaporize under a fume hood overnight, and oven dried the following day for 48 h at 60°C.

2.2 | Biochemical analyses

The moisture and ash contents of the meals were determined following the standard methods 934.01 and 942.05 of the Official Analytical Chemists (AOAC, 2010). An elemental analyzer model Eurovector EA3100 (Eurovector, Pavia,

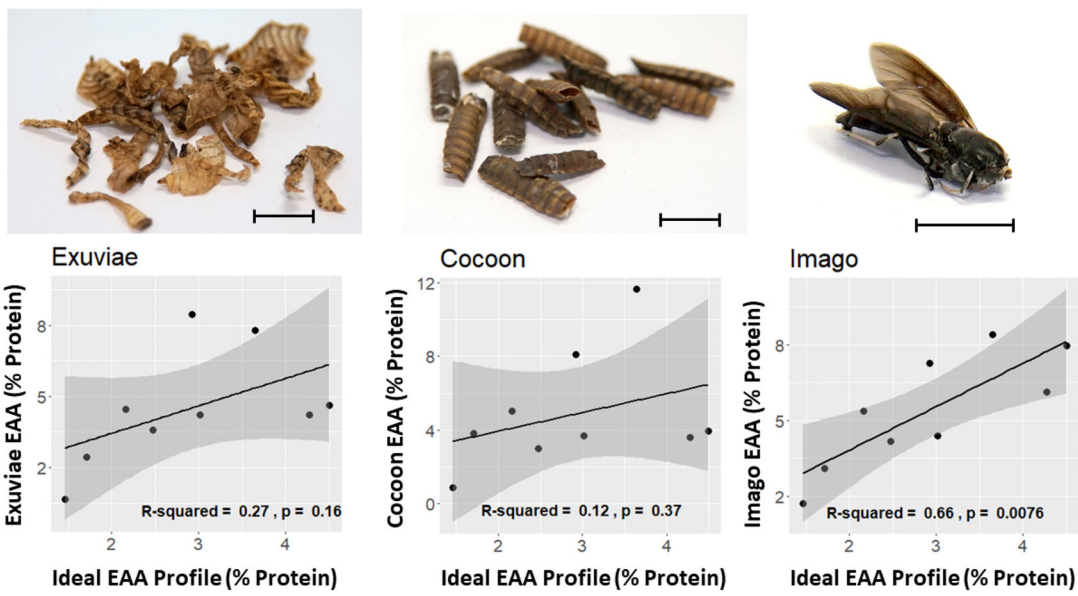

FIGURE 1 Images of the different by-products of industrial black soldier fly (*Hermetia illucens*) larvae production: exuviae of larvae, cocoons, and imagines (from the left to the right). The scale indicates a length of 1 cm. Graphics below each image show the linear correlation of the ideal essential amino acid (EAA) profile of dietary protein for shrimp (National Research Council, 2011) and the EAA contents in the respective raw material, expressed as % of protein.

Italy) was used to determine the carbon and nitrogen contents. Chitin was extracted after Percot et al. (2003) and determined gravimetrically. In brief, samples were demineralized with HCl (0.25 mol·L^{-1}) for 15 min at a solid to liquid ratio of 1:40 (w/v). Consecutive deproteinization was carried out with NaOH (1 mol·L^{-1}) at a ratio of 1:30 (w/v) for 24 h at 70°C. Subsamples of the extracted chitin were then combusted in a muffle furnace for 2 h at 600°C (method 942.05, AOAC (2010)) to correct the chitin contents for residual ash. The chitin bound nitrogen was subtracted from the total nitrogen content of the insect meal. The protein content was determined by multiplying the corrected nitrogen content by 6.25 (Dumas, 1831). Total lipid was measured gravimetrically after Folch et al. (1957) using dichloromethane/methanol (2:1, v/v) as solvent, as described by Postel et al. (2000) and Koch et al. (2023). Energy content was measured using a bomb calorimeter (Parr 6100, Parr Instrument Company, USA). A commercially available test kit (Boehringer, Germany) was used to determine the cholesterol content. Fatty acid (FA) and amino acid (AA) profiles were measured by a certified laboratory (LUFA Nord-West, Germany; methods: ASU L 13.00-26;2008-06, ASU L 13:00-27/2;2012-01 and VO (EG) 152/2009). Yttrium and minerals (P, K, Ca, Mg, Cu) were analyzed by an external commercial laboratory (Institute Dr. Nowak, Ottersberg, Germany) and measured via inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.3 | Digestibility trial

The digestibility trial was performed with test diets containing the insect meals and a reference diet. The reference diet was formulated with fishmeal and soy protein concentrate meal as primary protein sources (Table 1). Yttrium

TABLE 1 Ingredient composition of the reference diet.

Ingredient	Reference diet (g kg^{-1})
Fishmeal[a]	260
Soy protein concentrate[b]	220
Wheatmeal[c]	409
Fish oil[a]	20
Lecithin (soy)[d]	20
Gluten (wheat)[e]	25
Sodium alginate[f]	25
Vitamin and mineral premix[g]	10
Met-Met[h]	5
Yttrium oxide[i]	5
Cholesterol[j]	1

[a]Fishmeal: protein 64%, lipid 9%, and ash 21%; Bioceval GmbH & Co. KG (Cuxhaven, Germany).
[b]Protein 60%, lipid 1%, and ash 6%; Köster Marine Proteins GmbH (Hamburg, Germany).
[c]Protein 11%, lipid 2%, and ash 1%; Mühle Schlingemann e.K. (Waltrop, Germany).
[d]Louis Francois SAS (Croissy-Beaubourg, France).
[e]Kröner-Stärke GmbH (Ibbenbühren, Germany).
[f]BOS FOOD GmbH (Meerbusch, Germany).
[g]Vitamin and mineral premix (g kg^{-1} diet): retinyl acetate (3000 IU), cholecalciferol (3000 IU), dl-a-tocopherol 0.3, menadione 0.04, thiamine 0.03, riboflavin 0.03, pyridoxin-HCL 0.06, cyanocobalamin 0.15 (mg kg^{-1}), nicotinic acid 0.07, D-pantothenic acid 0.07, choline chloride 1, folic acid 6 (mg kg^{-1}), biotin 0.5 (mg kg^{-1}), vitamin C 0.125, inositol 0.3, iron 0.05, copper 0.04, manganese 0.02, zinc 0.075, iodine 2 (mg kg^{-1}), selenium 0.3 (mg kg^{-1}), cobalt 0.06 (mg kg^{-1}), and magnesium 0.3; Spezialfutter Neuruppin GmbH & Co KG (Neuruppin, Germany).
[h]Evonik Industries AG (Essen, Germany).
[i]Fisher Scientific GmbH (Schwerte, Germany).
[j]Merck KGaA (Darmstadt, Germany).

was added as indigestible marker at a concentration of 5 g kg^{-1}. All ingredients of the experimental diets were homogenously ground and sieved (<500 μm). The test diets contained the respective insect meals and the reference diet mash at a ratio of 3:7 (w/w). Water was added to the feed mixtures to achieve a water content of approximately 15%. The resulting dough was mixed thoroughly and pelleted using a PP120 pellet machine (Cissonius, Zehdenick, Germany) with a die hole diameter of 2.5 mm.

A batch of 600 shrimp (*L. vannamei*), weighing approximately 10 g each, were purchased from a nearby indoor shrimp farm (Neue Meere, Gronau (Leine), Germany) and transported to the facilities of the Center of Aquaculture Research of the Alfred-Wegener Institute, Helmholtz Centre for Polar and Marine Research in Bremerhaven, Germany. The shrimp were held in two 750-L tanks (tank dimensions: 1.2 m · 1.2 m · 0.6 m). The tanks were connected to a recirculation aquaculture system (RAS), which consisted of a mechanical drum filter, a biofilter, a protein skimmer, and an UV treatment device. Shrimp acclimatized to the facility conditions for 7 days. During this acclimation time, shrimp received commercial shrimp feed (38% crude protein, 11% fat) two times daily ad libitum.

After 1 week, 180 shrimp were randomly taken from the stock tank (mean weight 10.5 ± 2.0 g) and distributed equally into 15 separate 50-L tanks (tank dimensions: 0.50 m · 0.25 m · 0.45 m), all connected to a RAS as described above. Each tank was stocked with 12 individual shrimp with an average biomass of 125.7 ± 2.1 g per tank. The experimental diets (one reference diet, four test diets) were randomly allocated to the 15 tanks, resulting in three replicates per feed. Shrimp were fed three times daily at 08:00, 11:30, and 15:00 with an equivalent of 4.5% of the shrimp biomass per day. The amount of feed was adjusted weekly, assuming a shrimp growth of 2 g per week. Feces collection commenced 1 week after starting to feed the experimental diets. Therefore, 1 hour after the feed was applied, all tanks were cleaned of uneaten feed remains, feces, and shrimp exuviae. Two hours later, freshly produced feces were carefully collected from each tank using a fine meshed net and stored at −20°C. Feces collection was carried out at 11:00, 14:30, and 18:00. After 4 weeks, approximately 25 g of fecal material (wet weight) was collected per tank and the digestibility trial was terminated. Fecal material was freeze dried, manually homogenized using a mortar and pestle, and stored in a desiccator until further measurements.

The apparent nutrient digestibility coefficients (ADC) of protein, energy, carbon, and copper in the reference and test diets were calculated as proposed by Cho and Slinger (1979):

$$\mathrm{ADC}\,(\%) = 100 - \left[100\left(\frac{Y_{\mathrm{diet}}}{Y_{\mathrm{feces}}}\right)\cdot\left(\frac{N_{\mathrm{feces}}}{N_{\mathrm{diet}}}\right)\right],$$

with N being the considered nutrient concentrations, and Y the yttrium contents in the diet and feces samples, based on dry matter.

The apparent nutrient digestibility of the insect raw materials was determined after Bureau and Hua (2006):

$$\mathrm{ADC}_{(\mathrm{test\ ingredient})} = \mathrm{ADC}_{\mathrm{test\ diet}} + \left[(\mathrm{ADC}_{\mathrm{test\ diet}} - \mathrm{ADC}_{\mathrm{ref\ diet}})\cdot\left(\frac{0.7\cdot N_{\mathrm{ref}}}{0.3\cdot N_{\mathrm{test\ ingredient}}}\right)\right],$$

where N_{ref} is the nutrient concentrations of the reference diet mash and $N_{\mathrm{test\ ingredient}}$ being the nutrient contents of the tested insect-based ingredients (as is).

2.4 | Statistics

Datasets were tested for homogeneity of variance and normal distribution using the Bartlett's and Shapiro–Wilk tests, respectively. When parametric assumptions were met, group comparisons were made using one-way ANOVA. A Tukey post-hoc test was performed when statistically significant differences were detected ($p < 0.05$). If

homoscedasticity and normal distribution were not given, a Kruskal–Wallis test followed by a Nemenyi post-hoc test was made. Data and regression analysis were conducted with the software R (R Core Team, 2022).

3 | RESULTS

3.1 | Chemical composition

The initial moisture contents of exuviae, cocoons, and imagines were 35%, 30%, and 33%, respectively. Oven drying reduced the moisture content to approximately 5% in all materials, resulting in dry matter contents from 949 to 958 g kg^{-1} (Table 2). The crude protein content was lowest in exuviae with mean values of 317.1 ± 10.1 g kg^{-1} (mean ± SD). Cocoons showed approximately 10% higher crude protein levels, while the imago material had crude protein levels of 521.0 ± 8.7 g kg^{-1}. Defatting the imago meal significantly increased the crude protein content to almost 60% (596.7 ± 10.1 g kg^{-1}) and lowered the lipid content by 34%. Compared to imagines, exuviae and cocoons showed markedly lower total lipid levels. The energy content of the insect materials followed the same tendency as the lipid values: highest energy levels in the imago meal (26.9 ± 0.1 MJ kg^{-1}) and lowest in the cocoon meal (18.8 ± 0.7 MJ kg^{-1}). Chitin was found at similar concentrations in exuviae and cocoons, ranging from 139.2 ± 3.7 g kg^{-1} to 153.9 ± 6.1 g kg^{-1}, respectively. Imago meal comprised approximately one third of this amount. Defatting of the imago meal increased the chitin content by 19% to 60.4 ± 3.9 g kg^{-1}. All materials showed low amounts of cholesterol ranging from 1.2 ± 0.3 g kg^{-1}, in defatted, to 2.8 ± 0.3 g kg^{-1} in untreated imago meal. The ash content was similar in exuviae, imagines, and defatted imago meal. In contrast, the ash content in the cocoon meal reached values almost three times as high with 177.2 ± 1.0 g kg^{-1}. Cocoons also showed highest calcium concentrations of 41.8 g kg^{-1}, while exuviae and imagines contained only 6.2 and 0.6 g kg^{-1}, respectively (Table 3). The other tested macrominerals (phosphorus, potassium, and magnesium) were present at similar levels in the insect by-products, not exceeding 2% of the raw material. The copper content was highest in exuviae with 32 mg kg^{-1}, while the cocoons and imagines showed lower values of 22 and 16 mg kg^{-1}, respectively.

The insect materials contained high relative levels of amino acids that are essential for shrimp (EAA; Table 4). The essential amino acid index (EAAI) ranged from 1.5 to 1.9. Exuviae and cocoons were largely similar in the amino acid profiles, with some variations in leucine and glycine. Cocoons contained more than twice as much leucine, and approximately 60% more glycine than exuviae. Despite high levels of EAA, both materials showed a low correlation with the ideal shrimp dietary amino acid profile reported by the National Research Council (2011). The coefficient of

TABLE 2 Gross nutrient composition of exuviae, cocoons, imago, and defatted imago meal of *Hermetia illucens* expressed in g kg^{-1} "as is," unless otherwise indicated (values are presented as the mean ± standard deviation, $n = 3–5$).

Nutrient (g kg^{-1})	Exuvia	Cocoon	Imago	Imago-defat
Dry matter	949.3 ± 0.6^{a}	958.0 ± 1.2^{b}	951.6 ± 2.6^{ab}	950.7 ± 5.7^{ab}
Crude protein	317.1 ± 10.1^{a}	432.8 ± 25.1^{b}	521.0 ± 8.7^{c}	596.7 ± 10.1^{d}
Gross energy (MJ kg^{-1})	21.0 ± 0.6^{ab}	18.8 ± 0.7^{a}	26.9 ± 0.1^{b}	24.7 ± 0.1^{ab}
Total lipid	139.4 ± 2.6^{b}	63.8 ± 2.9^{a}	355.8 ± 1.7^{d}	234.4 ± 2.2^{c}
Chitin	139.2 ± 3.7^{c}	153.9 ± 6.1^{d}	50.6 ± 2.3^{a}	60.4 ± 3.9^{b}
Cholesterol	1.6 ± 0.3^{a}	1.5 ± 0.2^{a}	2.8 ± 0.3^{b}	1.2 ± 0.3^{a}
Ash	74.0 ± 0.2^{c}	177.2 ± 1.0^{d}	42.6 ± 0.5^{a}	46.5 ± 0.5^{b}

Note: Different superscript letters in the same row indicate significant differences ($p < 0.05$).

TABLE 3 Mineral content of exuviae, cocoons, and imago meal of *Hermetia illucens* as is expressed in g kg^{-1} unless otherwise indicated.

Mineral	Exuvia	Cocoon	Imago
Calcium	6.2	41.8	0.6
Phosphorus	8.2	10.6	9.0
Potassium	17.7	19.7	7.9
Magnesium	3.0	4.6	3.1
Copper (mg kg^{-1})	32.0	22.0	16.0

TABLE 4 Amino acid content (g kg^{-1}) of by-products derived from BSF larvae production.

Amino acid	Exuvia	Cocoon	Imago	Imago-defat
Arginine	10	11	24	29
Histidine	7	12	14	16
Isoleucine	11	16	22	25
Leucine	17	36	32	37
Lysine	11	12	30	35
Phenylalanine	9	9	18	20
Methionine	3	3	9	12
Threonine	10	12	18	22
Valine	18	25	28	32
Aspartic acid	19	23	42	50
Glutamic acid	30	31	50	59
Alanine	20	27	32	37
Cysteine	3	2	4	5
Glycine	20	33	22	25
Serine	12	16	17	20
Proline	19	23	25	28
Tyrosine	12	19	19	22
EAAI	1.5	1.5	1.8	1.9

Note: Essential amino acid index $(\mathrm{EAAI}) = \sqrt[n]{\frac{\mathrm{Arg}_p}{\mathrm{Arg}_I} \times \frac{\mathrm{His}_p}{\mathrm{His}_I} \times \frac{\mathrm{Iso}_p}{\mathrm{Iso}_I} \times \ldots \times \frac{\mathrm{Thr}_p}{\mathrm{Thr}_I}}$ with the subscript *p* referring to insect protein (exuvia, cocoon, imago, and imago-defat) and *I* referring to the ideal protein for penaeid shrimp (National Research Council, 2011).

determinations reached 0.27 and 0.12 for exuviae and cocoons, respectively (Figure 1). The imago meal contained higher concentrations of all amino acids (except leucine in cocoons) and reached an EAAI of 1.8. The correlation with the ideal dietary EAA was statistically significant ($r^2 = 0.66$, $p = 0.0076$).

All insect materials contained a high share of saturated fatty acids (SFAs; Figure 2), accounting from 47% of the total FAs in exuviae, to up to 83% in imagines. Lauric acid (C12:0) was the prevalent FA, accounting for 26% in exuviae, to more than 60% of the total FA contents in imagines. Other SFA, such as myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0), made up a small share of 2%–13% of the total FA. The absolute contents of mono- and polyunsaturated fatty acids (MUFA, PUFA) were highest in exuviae and imagines, while defatted imago meal and cocoons showed lower levels (Table 5). Oleic acid (C18:1(n-9)) was the predominant MUFA, while the PUFAs linoleic- (C18:2(n-6)) and linolenic acid (C18:3(n-3)) were found in varying levels throughout the tested materials. The

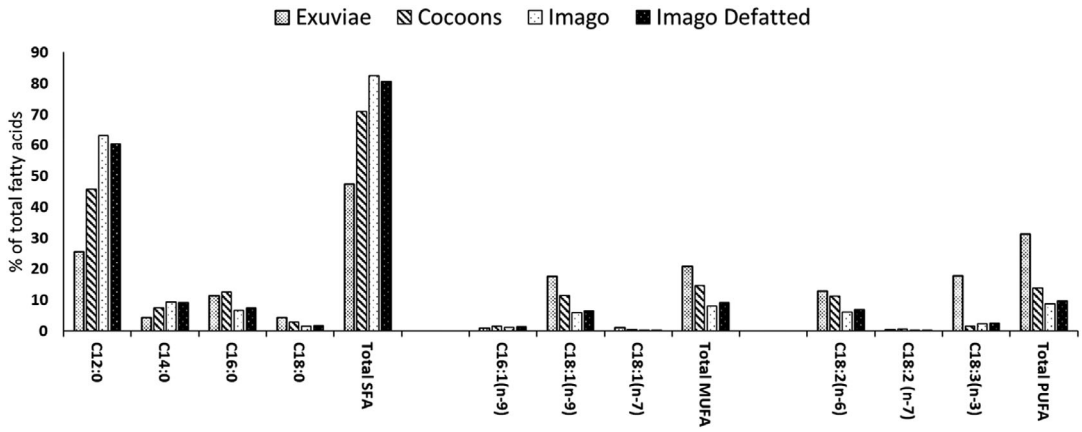

FIGURE 2 Fatty acid profile the different by-products of industrial black soldier fly (*Hermetia illucens*) larvae production: exuviae of larvae, cocoons, and imagines, expressed as % of total fatty acids.

TABLE 5 Fatty acid content (g kg^{-1}) of by-products derived from BSF larvae production.

Fatty acids	Exuvia	Cocoon	Imago	Imago-defat
Saturated fatty acids (*SFA*)				
C10:0	0.7	0.4	2.8	1.7
C12:0	30.7	21.1	169.6	117.6
C14:0	5.1	3.4	24.9	18.0
C16:0	13.7	5.8	18.1	14.5
C17:0	0.3	0.1	0.3	0.3
C18:0	5.2	1.4	4.1	3.5
C20:0	0.3	0.1	0.4	0.4
C22:0	0.3	0.1	0.4	0.3
Total SFA	56.6	32.6	221.5	157.1
Monounsaturated fatty acids (*MUFA*)				
C16:1(n-9)	1.2	0.7	3.4	2.7
C18:1(n-9)	21.1	5.2	15.7	12.9
C18:1(n-7)	1.4	0.2	0.6	0.6
C20:1(n-9)	0.4	0.2	1.0	0.7
Total MUFA	25.1	6.8	21.7	17.7
Polyunsaturated fatty acids (*PUFA*)				
C18:2(n-6)	15.5	5.2	16.5	13.4
C18:3(n-3)	21.4	0.7	6.6	5.1
Total PUFA	37.4	6.4	23.7	19.0

relative FA profile (Figure 2) showed that the contribution of MUFA and PUFA was highest in exuviae with 21% and 31% of the total FAs and decreased in cocoons to 15% and 14%. Imagines and defatted imagines contained the lowest percentage of MUFA and PUFA with 8%–10%.

3.2 | Digestibility

The apparent protein digestibility was lowest in exuviae with 19.6 ± 10.7% (Table 6). Both cocoons and imagines had a similar apparent protein digestibility of approximately 35% and 38%. The apparent energy and carbon digestibility of exuviae, cocoons, and imagines followed the same trend: higher values in the exuvia, lower apparent digestibility in imago, and lowest in cocoons. This pattern was also seen in the apparent copper digestibility, reaching values of 68.2 ± 12.9% in exuviae and 26.6 ± 14.6% in cocoons. Defatting of the imago meal resulted in an increase of all ADC. Apparently, 59.1 ± 4.4% of the protein of defatted imago meal was bioavailable for *L. vannamei*, leading to a digestible protein content of 370.7 g kg^{-1}. This was an increase of approximately 77%, compared to the digestible protein level of untreated imago meal with 209.6 g kg^{-1}. The digestible carbon and energy contents increased by 61% and 64%, when imago meal was defatted, reaching absolute digestible values of 227.9 g kg^{-1} and 13.8 MJ kg^{-1}.

4 | DISCUSSION

Exploring suitable nutrient sources from by-products for aquaculture feeds is essential for the development of sustainable aquaculture. BSF larvae are a valuable animal feed ingredient suitable for terrestrial and aquatic animal nutrition. There are numerous biological by-products remaining during BSF larvae production and this study reports initially the detailed chemical composition of these by-products and the bioavailability of key nutrients for *L. vannamei*. These insect remains contain considerable amounts of crude protein (30%–60%) and low (<10%) to moderate (18%) amounts of ash. A high share of EAA and the presence of biomolecules such as cholesterol and chitin, as well as micro-minerals like copper, make these raw materials especially interesting as ingredients for shrimp feed.

The crude protein content of exuviae and cocoons is comparable to that of plant-based ingredients such as pea, canola, lupin, and peanut meal, which have been successfully tested as alternative protein ingredients in shrimp feeds (Cruz-Suarez et al., 2001; Liu et al., 2012; Smith et al., 2007; Weiss et al., 2020). The imago life stage of the BSF showed a crude protein content similar to that of the BSF larvae, which varies between 400 and 600 g kg^{-1} (Cummins Jr et al., 2017; Richardson et al., 2021; Spranghers et al., 2017). The amino acid profile of imago largely resembles that of the BSF larvae with high levels of EAA such as leucine, isoleucine, and lysine (Cummins Jr

TABLE 6 Apparent digestibility coefficients (ADC) for energy, carbon, protein, and copper of black soldier fly larvae production residues and the calculated amount of the respective digestible nutrient (on dry matter basis, values are expressed as mean ± standard deviation).

Nutrient ADC (%)	Exuvia	Cocoon	Imago	Imago-defat
Protein	19.6 ± 10.7^{a}	35.4 ± 16.6^{ab}	38.3 ± 8.3^{ab}	59.1 ± 4.4^{b}
Energy	44.4 ± 9.2^{bc}	24.4 ± 8.3^{a}	29.9 ± 1.4^{ab}	53.1 ± 3.0^{c}
Carbon	45.7 ± 13.2	25.1 ± 10.2	27.7 ± 14.1	49.2 ± 5.0
Copper	68.2 ± 12.9^{b}	26.6 ± 14.6^{a}	36.7 ± 15.9^{ab}	N/A
Digestible nutrient				
Protein (g kg^{-1})	65.6	160.0	209.6	370.7
Energy (MJ kg^{-1})	9.8	4.7	8.4	13.8
Carbon (g kg^{-1})	227.9	113.2	141.7	227.9
Copper (mg kg^{-1})	23.0	6.1	6.2	N/A

Note: Values with different superscript letters are statistically different ($p < 0.05$).

et al., 2017; Oteri et al., 2021; Spranghers et al., 2017). All insect by-products potentially provide sufficient EAA needed for shrimp nutrition as indicated by the high EAAI. However, the low correlation between EAA in exuviae and cocoons versus the ideal EAA of dietary protein implies some amino acid (AA) imbalances, if these resources were used as single AA sources in shrimp diets. In contrast, the significant correlation of EAA between imago meal and the ideal protein requirements suggests that imago meal is a more appropriate protein source for *L. vannamei*.

The different AA composition of exuvia and cocoon meals compared to imago meal could be explained by the predominantly structural and chitin-bound proteins in these exoskeleton-based materials. Exoskeletons of insects primarily consist of sclerotized chitin and protein that act as a protective barrier against the external environment (Willis, 1987). Proteins associated with exoskeletons show specific AA compositions, which, in turn, are linked to the physio-chemical property of the cuticle in different life stages of insects (Andersen et al., 1995; Kumari et al., 1995). Chitin is another major biomolecule in insect exoskeletons and accounts for 20% and 40% in *H. illucens* exuviae of different life stages (Hahn et al., 2018). The values for exuviae and cocoons obtained in the current study are lower (14%–15%). This discrepancy might be because of natural variation in the biological material, impurities of the raw materials, and/or differences in the chitin determination methods. The nutritional relevance of chitin and its derivatives for penaeid shrimp remains under debate (Akiyama et al., 1989; Brol et al., 2021; Clark et al., 1993; Fox, 1993; De los Santos-Romero et al., 2017). Nevertheless, excellent aquafeed ingredients such as shrimp head meals contain chitin in the range of 90–178 g kg^{-1} (Fox et al., 1994; Fricke et al., 2022; Synowiecki & Al-Khateeb, 2000) and *L. vannamei* possess chitinases that indicate their capability to digest chitin (Sotelo-Mundo et al., 2009).

Liu et al. (2017) found changes in the crude lipid content between 5% and 32% of the dry mass throughout the different life stages of *H. illucens*. The lipid content increased steadily up to 28% during the feeding phase of the larvae, but decreased again drastically to 7% in late pupal stage. After metamorphosis to imago, the fat content reached highest values of more than 30%, presumably accumulating energy reserves for reproduction (Liu et al., 2017). These variations in lipid content are consistent with our findings, showing intermediate lipid values in exuviae, low contents in cocoons, and highest in the imago.

The changes in FA composition throughout the lifespan of *H. illucens* are also coherent with the FA profile of BSF production residues that originate from the respective life stage. While SFAs are predominant over the whole lifespan, unsaturated FAs, such as oleic acid (C18:1(n-9)), linoleic acid (C18:2(n-6)), and linolenic acid (C18:3(n-3)), make up a larger share in early larval stages (Liu et al., 2017). Exuviae from larvae stages also showed highest shares of unsaturated FAs.

Shrimp do not tolerate high levels of dietary lipids (National Research Council, 2011). Therefore, high fat insect meals are often defatted to achieve a more suitable nutrient profile (Cummins Jr et al., 2017; Motte et al., 2019; Oteri et al., 2021). Defatting of imago meal in our study reduced the total lipid content without altering the FA profile. While the defatting also resulted in a decrease in cholesterol and the overall energy content, non-fat nutrients such as protein, chitin, and ash increased accordingly.

The apparent digestibility of protein, energy, and carbon increased in defatted imago meal. It seems that a high lipid content negatively affects nutrient bioavailability. This is in line with Glencross et al. (2002), who found significantly reduced lipid digestibility in *Penaeus monodon* when fed diets containing 135 g kg^{-1} of total lipid.

The apparent nutrient digestibility of exuviae, cocoons, and imagines did not exceed 50% (except copper in exuviae). In comparison, the apparent protein digestibility of BSF larvae meals in *L. vannamei* range between 72% and 85% (Li et al., 2022; Shin & Lee, 2021). The protein of other insect species such as the mealworm (*Tenebrio molitor*) is even less efficiently digested by *L. vannamei*, with values ranging from 42% to 57% (Li et al., 2022; Panini et al., 2017). This is in contrast to the findings of Shin and Lee (2021), who reported ADC protein values of 84% for mealworm meal. The mealworm used in the study of Shin and Lee (2021) differed in proximate composition, containing less protein and less lipid compared to the mealworm used in the other studies (Li et al., 2022; Panini et al., 2017). It appears that the bioavailability of the nutrients in insect meals is strongly affected by insect taxa, life-stage of the insect, and the gross nutrient composition of the material. For instance, chitin has been assumed to negatively influence nutrient digestibility and is sometimes classified as anti-nutritional and indigestible fiber (Barroso

et al., 2014; Kroeckel et al., 2012; Shiau & Yu, 1998, 1999). Despite the presence of chitinolytic enzymes in the digestive tract of *L. vannamei* (Huang et al., 2010), chitin seems to be poorly digested with ADCs of around 30% (Clark et al., 1993; Shin & Lee, 2021). Higher concentrations of undigested chitin might therefore interfere with the absorption of other nutrients, similar to the effects of dietary non-starch-polysaccharides (Glencross et al., 2012). Furthermore, proteins present in insect exoskeletons that are sclerotized or bound to chitin could be less accessible for digestion as pointed out by Panini et al. (2017). This might explain the low ADC protein measured in chitin-rich exuviae and cocoons, which are primarily composed of exoskeletal material.

Glencross et al. (2002) observed that the digestibility of FA in *P. monodon* increased with the share of unsaturated FAs and with shorter FA chain lengths. The low energy digestibility observed in all tested materials of the current study is probably caused by the low digestibility of the SFAs that are predominant in the insect materials. The slightly higher apparent energy and carbon digestibility in exuviae could therefore be explained by the higher share of well-digestible MUFA and PUFA.

Insects comprise a large variety of antimicrobial compounds such as peptides, FAs, and chitin/chitosan (Lagat et al., 2021; Saadoun et al., 2022; Saviane et al., 2021). Improved pathogen resistance and immunological parameters were reported for *L. vannamei* when fed with *H. illucens* larvae and *T. molitor* (Choi et al., 2018; Motte et al., 2019; Richardson et al., 2021). Insect-based immunostimulants could provide a viable and safe alternative to antibiotics (Saadoun et al., 2022), which have been banned in livestock feed in the European Union (European Commission, 2003). Exuviae, cocoons, and imagines contain biologically relevant compounds (e.g., chitin, lauric acid, cholesterol, copper), which may promote growth, health, and disease resistance. Dietary supplementation of chitin and its derivatives was shown to improve performance in penaeid shrimp (Brol et al., 2021; Niu et al., 2013; Shiau & Yu, 1998). Copper is an essential micronutrient for crustaceans, needed for many metabolic processes and essential in hemocyanin synthesis (Culotta et al., 2006; Depledge, 1989; National Research Council, 2011). Cholesterol is considered indispensable in shrimp diets, and supplementation of purified cholesterol is one of the key drivers in feed production cost (Kanazawa et al., 1984; Smith et al., 2001; Zhang et al., 2019). Especially in diets increasingly replacing marine ingredients with plant-based alternatives, by-products of BSF larvae production could be included as dietary cholesterol source.

The ADC of copper was highest in exuviae, resulting in 23 mg kg^{-1} of bioavailable copper. This value seems high in comparison with the other insect materials investigated in this study, but is only about half the amount of digestible copper found in brown shrimp processing remains (Fricke et al., 2022). Phosphorus was present in similar levels across the tested insect materials and were slightly higher than in early BSF prepupa (Liu et al., 2017). The high calcium content found in cocoons indicates a high degree of calcification, thus explaining the rigid structure of this material. Information on the dietary requirements of minerals in shrimp is scarce and hard to determine because marine crustaceans can absorb certain minerals directly from the ambient seawater (National Research Council, 2011). Yet, information on mineral contents in feed ingredients is relevant to formulate high precision feeds and account for mineral interactions, for example, appropriate calcium/phosphorous ratios, to ensure sufficient phosphorous availability (Davis et al., 1993).

5 | CONCLUSIONS

Despite promising chemical compositions of BSF larvae production by-products, the bioavailability of key nutrients is limited for *L. vannamei*. Defatting of imago meal resulted in a significantly increased nutrient digestibility. These findings, along with an improved nutrient profile, encourage further investigation of defatted imago meal as an alternative protein source in shrimp feeds. Exuviae and cocoons, with high chitin, copper, and cholesterol contents are as well interesting candidates as functional ingredients, rather than replacing macronutrients in aquafeeds. Along with growing industrial production of insects, the availability of these novel by-products will increase accordingly,

ensuring a year-round steady supply. These novel resources can represent sustainable and efficient feed ingredients in the future.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no personal nor financial interests that could have appeared to influence the work of this study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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