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RESEARCH ARTICLE

Batch-to batch variation in nutrient digestibility of black soldier fly larvae meals in rainbow trout

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Abstract

The aim of this study was to evaluate the apparent digestibility coefficients (ADCs) of four batches of black soldier fly (BSF) meal, named BSF1, BSF2, BSF3 and BSF3, produced by the same company over one year, in rainbow trout (*Oncorhynchus mykiss*). To assess nutrients and amino acids ADCs, each insect meal was mixed with a high-quality reference diet in a 30:70 ratio on as is basis, including celite as inert marker, and pelleted. The reference diet was also used as control. The ADCs were calculated based on the data collected and determined during an *in vivo* experiment. A total of 240 fish were randomly divided into 250-L cylindroconical tanks supplied in flow-through open and Choubert systems (3 replicates/treatment). Data were analysed by One-Way ANOVA (SPSS V20.0.0.; $P \leq 0.05$). Globally, the four BSF meals showed a high ADC value, between 82.6 and 100%. No statistically significant differences were observed in the ADCs of dry matter, crude protein, ether extract and gross energy, while some differences were observed in the amino acid profile ($P < 0.05$). Generally, fish fed on BSF1 displayed reduced digestibility for histidine, isoleucine, leucine, valine, alanine, proline, and glycine compared to BSF2 and BSF4 meals ($P < 0.05$), while the BSF3 group showed intermediate results ($P > 0.05$). Despite the good digestibility of nutrients for all the insect mealsthe implementation of standardized production is important to have a standardize BSF meal capable of meeting market demands.

Keywords

amino acids – feed ingredients – *Hermetia illucens* – partially-defatted – salmonids – sustainability

1 Introduction

The causes that have led to the search of alternative protein sources in aquafeed are different and generally known: the population growth, the increase in animal production and the environmental and economic costs of the conventional protein feed-ingredients (e.g. soybean and fish meals). The production and supply of soybean and fish meal is actually absorbed by the market demand, thus making it challenges to accommodate future increases (EUMOFA, 2021; Ritchie, 2021). Since 1960 until now, the annual soy production has increased by 300 million tonnes (Our World in Data, 2023). This increase in production was made possible through the improvements in the yields and the extension of land use. In the soy scenario, the expansion of croplands is clearly the main driver of the exponential production that has taken place (Ritchie, 2021). Considering the same period, fish meal has undergone a fluctuating production, overall registering an increase of only 7

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million tonnes (Pauly *et al.*, 2020). The different production patterns for the two protein sources are the result of their availability. Concerning soybean meal, a production increase is still possible, considering that natural territories can still be changed into cultivable lands, while marine resources are already limited. This aspect becomes evident when comparing aquaculture production, which has increased by 104 million tonnes between the 1960s and the present day, with fish meal production (Pauly *et al.*, 2020). Since marine resources are not rapidly renewable, the fish meal production trend cannot follow the aquaculture expansion. Among the alternative protein that are under evaluation by research and companies, insect meal seems to well combine both the nutritional value and the reduced environmental impact sought by the market. The inclusion of insect meals in aquafeeds began to receive attention from the 2000s, with the use of house fly mainly (Ajani *et al.*, 2004; Ogunji *et al.*, 2008). Nowadays, the interest on house fly as feed ingredient is reduced when compared with other insect species – specifically black soldier fly (BSF) (van Huis *et al.*, 2020). Since the BSF life cycle is fully applicable to the circular economy concept, it is one of the most promising species for feed production (van Huis and Gasco, 2023) and, considering that the aquafeed sector was the first in which the insect derived-products were regulated in Europe (Regulation (EU) 2017/893), several studies focused on the growth performance and/or nutrient digestibility of the diet including BSF meal (Weththasinghe *et al.*, 2021; Mohan *et al.*, 2022). The apparent digestibility coefficient (ADC) of a specific nutrient indicates the proportion to which the nutrient is digested by an animal. This parameter is of crucial importance to formulate diets capable to cover the nutritional requirements of the animal. Moreover, it also allows to prevent environmental pollution caused by an excessive release of nutrients (Gasco *et al.*, 2023). While many papers have reported on the digestibility of diets containing BSF meals (Biasato *et al.*, 2022; Caimi *et al.*, 2021; Guerreiro *et al.*, 2021; Karapanagiotidis *et al.*, 2023; Moutinho *et al.*, 2022), the nutrient digestibility of the BSF meal as ingredient for the main fish species reared in Europe, such as European sea bass (Basto *et al.*, 2020), Atlantic salmon (Radhakrishnan *et al.*, 2022) or rainbow trout (Dumas *et al.*, 2018; Gasco *et al.*, 2022), has been little investigated. Due to the wide variety of waste on which BSF can grow and the different processing methods available to obtain insect-derived products, the composition of the meal generally changes among producers. Moreover, considering that the by-products production is in some cases linked to a seasonality, the achievement of a standardize meal – in terms of nutrients – could be difficult also when a unique producer is considered. For these reasons, this study aimed to determine the apparent digestibility coefficients (ADCs) of four partially defatted BSF meals produced in different period of the year by the same producers in order to evaluate their nutritional stability and variation in digestibility.

2 Materials and methods

The trial was performed at the Experimental Facility of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Turin (Italy) after being approved by the Ethical Committee of the University of Turin (Italy) (protocol n° 15741). The experimental protocol was draw up following the European Directive on the on the protection of animals used for scientific purposes (2010/63/EU).

Insect meals and experimental diets

Both the larvae and the partially defatted meals were produced by Hermetia Baruth GmbH company in four different period in one year (December 2019, and February, May and October 2020 – named BSF1, BSF2, BSF3 and BSF4, respectively). According to the information obtained from the producer, BSF1, 3 and 4 derived from larvae grown on the same substrate, while the BSF2 was different. However, all the used substrates were composed by vegetal by-products authorized by the European regulation 2022/1104 (Commission Regulation, 2022). To obtain meal, the larvae were dry processed. Once received, the meals were stored at room temperature in a dry place and a representative sample of 100 g was used to determine the proximate composition, gross energy (GE), and the amino acid (AA) profile.

Each insect meal was added to a high-quality reference diet (Table 1) in 30:70 ratio on as is basis (Bureau *et al.*, 1999). To evaluate the digestibility of the insect meals, the indirect approach was carried out by including an acid insoluble ash (Celite; Fluka, St. Gallen, Switzerland) as inert marker in the reference diet. The mixtures were thoroughly blended and pelleted by meat grinder with a 3.0 mm mesh, then the feed pellets were dried (50 °C and 48 h) and stored in a dark and cold (4 °C) chamber prior to use.

Fish and trial set-up

The trial was performed using rainbow trout (*Oncorhynchus mykiss*) supplied from a private fish hatchery

TABLE 1 Ingredients (g/100 g as is), proximate composition (g/100 g of dry matter, unless otherwise stated), and gross energy content (MJ/kg on dry matter) of the reference diet

Ingredients (g/100 g, as is)	Reference diet
Fish meal	50
Soybean meal	16.5
Wheat meal	12
Starch gealtinized	8.0
Wheat gluten meal	2.0
Fish oil	10
Premix vit/min	0.5
Celite®	1.0
Chemical composition (g/100 g, DM)	
DM (g/100 g)	88.90
CP	53.68
EE	13.51
Ash	11.72
NFE	21.10
GE	19.71

DM = dry matter; CP = crude protein; EE = ether extract; NFE = nitrogen-free extracts; GE = gross energy. Nitrogen free extracts were calculated as: 100 – (CP% + EE% + ash%).

(Troticoltura Bassignana, Cuneo, Italy) with similar initial body weight (219.2 g ± 9.3 g). The ADCs were obtained through an *in vivo* experiment into 250-L cylindroconical tanks supplied in flow-through open system, with water inflow of 8 L/min, connected to an Artesian well water (constant temperature of 13 ± 1 °C). Specifically, 240 fish were randomly divided in groups of 16 individuals and placed inside of each tank (3 replicates/treatment). The fish were acclimatised to the tanks for a 14-day period during which they were fed a commercial diet. An additional 10-day period was then dedicated to adapting the animals to the experimental feeds. Fish were fed by hand until visual satiety twice a day (8:00 and 14:00) and seven days per week in both the acclimatisation period and trial. The faeces collection lasted 3 weeks, and they were collected through a continuous automatic device (Choubert *et al.*, 1982). For each tank, all the faeces collected during the trial were pulled together and kept frozen (–20 °C), then the samples were freeze-dried to perform the chemical analyses.

Chemical analyses

Before performing the chemical analyses, the diets and the freeze-dried faeces were ground (Retsch, GM 200). The dry matter (DM; AOAC #934.01), the crude protein (CP; AOAC #984.13; 5.62 nitrogen to protein (N-P) conversion factor for insect meals [Janssen *et al.*, 2017]) and the ash (AOAC #942.05) were determined by the International AOAC (AOAC, 2000), and the ether extract (EE; AOAC #2003.05) by the International AOAC (AOAC, 2003). The chitin content was analysed following the methodology reported by Woods *et al.* (2020). The GE was determined using adiabatic calorimetric bomb (C7000; IKA, Staufen, Germany). Analysis of total AAs (not including cysteine and tryptophan) of the feed and faeces was carried out by ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC system) coupled with a UV detector (Espe *et al.*, 2014; Liland *et al.*, 2017). Wet, powdered samples containing 30-40 mg of protein was hydrolysed in 6 M HCl at 110 °C for 22 hours. Prior to hydrolysis, 3.125 mM Norvaline (Sigma-Aldrich, St. Louis, MO, USA) was added as internal standard, and 0.1 M Dithiothreitol (DTT, Sigma-Aldrich) as an antioxidant agent to protect methionine from degradation during acid hydrolysis. As a further protective step, sample tubes were topped up with nitrogen gas. After hydrolysis, samples were cooled to room temperature and centrifuged in a vacuum centrifuge until they are completely dry. Subsequently, the residue was diluted in MilliQ-Plus water and filtered through a syringe-driven filter. Before the instrumental analysis, a derivatisation agent (AccQ.Tag™, Waters, Milford, MA, USA) was added to each sample. Finally, amino acids were separated by UPLC (column: Aquity UPLC BEH C18 1.7 μM, Waters, flowrate 0.7 mL min^{-1}) and results integrated by Empower 3 (Waters).

ADC equations and statistical analyses

The ADCs of the tested insect meals were calculated starting from the ADCs of the diets and expressed as a percentage, following the equations indicated by Bureau *et al.* (1999) and specifically:

$$ADC = 1 - [(F/D) \times (Di/Fi)]$$

where: F = % nutrient (or kJ g^{-1} GE) in the faeces; D = % nutrient (or kJ g^{-1} GE) in the reference or experimental diet; Di = % inert marker in the diet; Fi = % inert marker in the faeces.

The ADC of DM was calculated as:

$$ADC_{DM} = 100 \times [1 - (Di/Fi)]$$

The ADCs of the nutrients and energy of each of the tested BSF meals were obtained as:

$$ADC_{ing} = ADC_{test} + [(ADC_{test} - ADC_{ref}) \times ((0.7 \times D_{ref}) / (0.3 \times D_{ing}))]$$

where: ADC_{test} = ADC (%) of the experimental diet; ADC_{ref} = ADC (%) of the reference diet; D_{ref} = g $100g^{-1}$ nutrient (or MJ kg^{-1} GE) of the reference diet (DM basis); D_{ing} = g 100 g^{-1} nutrient (or MJ kg^{-1} GE) of the test ingredient (DM basis).

The digestible essential amino acids (EAA; mg/g DM) were calculated by multiplying the content of each EAA by the corresponding ADC.

Data was analysed using the IBM SPSS Statistics software (V20.0.0.; IBM, Armonk, NY, USA). For all the parameters recorded the statistical unit was the tank. The assumptions of normality distribution and equal variances were determined using Shapiro-Wilk and Levene's homogeneity of variance tests, respectively. The data analyses of the nutrients and GE digestibility was performed through One-Way ANOVA, by expressing the results as mean and pooled standard error of the mean (SEM). The level of significance considered was ≤0.05.

3 Results

Insect meals

Table 2 illustrates the chemical composition and GE of the four BSF meals used in this trial. Among these, BSF4 exhibited the lowest DM value, while BSF3 showcased the highest. The CP content varied from 56.02 g/100 g DM (BSF2) to 61.88 g/100 g DM (BSF2), with BSF2 recording the minimum and BSF1 the maximum. The EE content displayed an inverse pattern to CP, with BSF1 having the lowest value and BSF2 the highest. The BSF3 demonstrated the highest ash content, whereas BSF1 had the lowest. Chitin content ranged from 7.82 g/100 g DM (BSF2) to 8.56 g/100 g DM (BSF1). In terms of GE, the lowest value was found in BSF3 meal.

The AAs profiles of the BSF meals are illustrated in Table 3. The BSF1 and BSF2 exhibited the highest EAA values, with the only exception of leucine that was higher in BSF4 than BSF2. For all the essential AA (EAA), BSF3 showed the lowest values. Specifically, BSF1 had the greatest quantities of histidine, isoleucine, leucine, methionine, valine, and threonine, while arginine and phenylalanine were the most represented in BSF2. All the BSF meals showed a higher value of total non-EAAs when compared to total EAAs. The highest value of total AAs was reported by BSF1, while a 13.5% lower content was present in BSF3.

Insect meal digestibility

The nutrients and GE digestibility are illustrated in Table 4. For all the parameters evaluated, no differences were observed among treatments ($P > 0.05$), but for DM, CP and EE parameters a statistical trend was denoted (0.056, 0.062 and 0.058, respectively). Specifically, the ADCs of DM and CP tend to be higher in BSF4 when compared to the other meals, while the ADC of EE showed a propensity for being highest in both BSF3 and BSF4.

Table 5 shows the digestibility of the AAs. Overall, the ADC values recorded were high for all the AAs. Among the EAAs, histidine, isoleucine, leucine, valine, and threonine showed statistical differences among the insect meals. The digestibility of histidine, isoleucine, and leucine, was higher in BSF2 and BSF4 groups than BSF1 ($P < 0.05$ and $P < 0.01$, respectively), while BSF3 showed an intermediate value ($P > 0.05$).

No differences in terms of valine digestibility were observed between BSF2 and BSF4 ($P > 0.05$), and BSF2 was also comparable to BSF3 ($P > 0.05$), while BSF1 and BSF3 differed among meals, with BSF1 showing the lowest ADC value ($P < 0.001$).

Concerning non-EAAs, the alanine ADC was the highest in BSF4 and the lowest in BSF1 ($P < 0.001$), respectively, while BSF2 and 3 displayed intermediate and statistically equal values ($P > 0.05$). Proline and glycine showed a similar pattern than valine and histidine, respectively.

Table 6 reports the EAAs nutritional value of each BSF meal, expressed in terms of digestible EAAs content (mg/g DM), as well as the EAA requirements of rainbow trout (NRC, 2011). Even if we did not perform a statistical analysis among meals, it can be said that BSF2 exhibited the highest values for digestible AAs, with the only exception of leucine that was similar in BSF 1 and BSF2.

4 Discussion

To the best of the authors' knowledge, the present study is the first one that focuses on the evaluation of BSF meals produced on a large-scale farm over a one-year period of time. Fish meal represents the optimal protein source largely used in the past in aquafeed. However, the well-known issues related to its use pushed the research for alternative raw materials, and nowadays, accent is not only on nutritional value but also

TABLE 2 Proximate composition (g/100 g DM, unless otherwise stated), and gross energy (MJ/100 g DM) of the four BSF meals

Items	BSF1	BSF2	BSF3	BSF4
DM (g/100 g)	96.57	98.13	98.53	95.11
CP	61.88	56.02	56.28	60.39
EE	4.35	6.44	5.92	4.50
Ash	8.32	9.92	10.68	10.25
Chitin	8.56	7.82	7.84	8.48
NFE[1]	16.89	19.81	19.29	16.37
Gross energy (MJ/100 g DM)	20.84	20.60	20.20	20.85

BSF1 = black soldier fly meal produced in December 2019; BSF2 = black soldier fly meal produced in February 2020; BSF3 = black soldier fly meal produced in May 2020; BSF4 = black soldier fly meal produced in October 2020; CP = crude protein; EE = ether extract; FM = fish meal; NFE[1] = Nitrogen free extracts = calculated as: 100 – (CP% + EE% + ash%).

TABLE 3 Amino acids profile (mg/g DM) of the four BSF meals

	BSF1	BSF2	BSF3	BSF4
Essential amino acids				
Arginine	29.20	29.45	25.27	28.49
Histidine	18.74	18.65	15.43	17.87
Isoleucine	26.92	26.70	22.73	25.65
Leucine	43.49	40.76	36.54	41.01
Methionine	10.87	10.80	8.83	9.99
Lysine	35.21	34.65	28.93	33.65
Valine	38.31	37.71	32.48	36.80
Threonine	25.06	24.76	22.13	24.71
Phenylalanine	26.72	26.80	23.14	25.65
$\sum$ EAA	245.53	250.28	215.47	243.82
Non-essential amino acids				
Alanine	43.49	42.80	41.61	46.26
Proline	37.28	36.69	32.48	37.85
Glycine	36.24	35.67	29.84	33.65
Tyrosine	40.39	39.74	33.49	39.95
Aspartic Acid	57.99	57.07	48.72	55.72
Glutamic Acid	67.31	65.22	69.92	66.24
Serine	27.13	27.00	23.75	26.92
Hydroxy-Proline	<0.6	<0.6	<0.6	<0.6
Taurine	<0.6	<0.6	<0.6	<0.6
$\sum$ non-EAA	309.83	304.19	272.81	306.59
$\sum$ AA	564.36	554.47	488.28	550.42

AA = amino acids; BSF1 = black soldier fly meal produced in December 2019; BSF2 = black soldier fly meal produced in February 2020; BSF3 = black soldier fly meal produced in May 2020; BSF4 = black soldier fly meal produced in October 2020; EAA = essential amino acid.

on sustainability (Colombo *et al.*, 2023). In comparison to other partially defatted BSF meals, the average DM content of the four insect meals was equal to 97.1 g/100 g, surpassing values reported in previous studies, such as 94.5% (Kishawy *et al.*, 2022), 94.8% (Gasco *et al.*, 2022) and 92.1% (Dumas *et al.*, 2018), thus rendering them more stable during storage (Kamau *et al.*, 2018). Nutrient quality can be affected by the meal water content, since the BSF meal can be stored for a period of 200-220 days at 25 °C, considering a moisture content of approximately 5% (Kamau *et al.*, 2018). Water can namely facilitate microbial, biochemical, and chem-

TABLE 4 Apparent digestibility coefficients (ADC, %) of dry matter, crude protein, ether extract, and gross energy of the four BSF meals

Items	BSF1	BSF2	BSF3	BSF4	*P*-value
ADC_{DM}	82.66 ± 2.60	85.20 ± 2.24	82.93 ± 3.38	90.10 ± 3.68	0.056
ADC_{CP}	84.93 ± 1.24	89.61 ± 0.25	84.68 ± 1.44	91.39 ± 3.58	0.062
ADC_{EE}	96.94 ± 2.09	96.25 ± 2.32	100.0 ± 0.21	99.76 ± 0.36	0.058
ADC_{GE}	87.63 ± 2.46	91.36 ± 1.81	88.74 ± 1.55	90.17 ± 1.21	0.142

ADC_{DM} = apparent digestibility coefficient of dry matter; ADC_{CP} = apparent digestibility coefficient of crude protein; ADC_{EE} = apparent digestibility coefficient of ether extract; ADC_{GE} = apparent digestibility coefficient of gross energy; BSF1 = black soldier fly meal produced in December 2019; BSF2 = black soldier fly meal produced in February 2020; BSF3 = black soldier fly meal produced in May 2020; BSF4 = black soldier fly meal produced in October 2020.

TABLE 5 Apparent digestibility coefficients (ADC, %) of amino acids

Items	BSF1	BSF2	BSF3	BSF4	*P*-value
Essential amino acids					
Arginine	99.06 ± 0.89	98.86 ± 1.03	96.33 ± 1.08	97.55 ± 2.68	0.208
Histidine	92.59 ± 0.17^{b}	95.99 ± 1.11^{a}	93.92 ± 1.88^{ab}	96.13 ± 0.64^{a}	0.014
Isoleucine	92.68 ± 0.55^{b}	96.17 ± 0.62^{a}	93.83 ± 1.76^{ab}	96.63 ± 0.84^{a}	0.005
Leucine	92.61 ± 0.62^{b}	96.53 ± 0.80^{a}	93.81 ± 1.61^{ab}	96.28 ± 0.92^{a}	0.005
Methionine	97.35 ± 0.93	97.44 ± 2.74	95.66 ± 1.99	97.84 ± 1.10	0.516
Lysine	96.03 ± 0.98	97.77 ± 0.58	95.69 ± 0.86	98.03 ± 1.53	0.058
Valine	90.04 ± 0.29^{c}	94.23 ± 0.48^{ab}	92.80 ± 1.46^{b}	96.10 ± 0.65^{a}	0.000
Threonine	93.53 ± 0.79	96.28 ± 0.67	93.66 ± 1.77	96.64 ±	0.014
Phenylalanine	95.86 ± 0.38	97.25 ± 0.74	94.83 ± 1.73	97.46 ±	0.067
Non-essential amino acids					
Alanine	89.58 ± 0.56^{c}	93.98 ± 0.42^{b}	93.13 ± 0.84^{b}	96.94 ± 1.69^{a}	0.000
Proline	89.77 ± 0.15^{c}	94.35 ± 0.48^{ab}	92.55 ± 0.94^{b}	96.49 ± 1.87^{a}	0.000
Glycine	83.78 ± 0.34^{b}	90.64 ± 0.96^{a}	87.66 ± 1.21^{ab}	90.09 ± 2.99^{a}	0.004
Tyrosine	93.96 ± 0.30	95.60 ± 0.42	94.21 ± 1.43	94.71 ± 1.83	0.403
Aspartic Acid	95.34 ± 1.74	96.26 ± 0.24	93.56 ± 1.62	96.68 ± 0.99	0.018
Glutamic Acid	94.49 ± 0.92	96.37 ± 0.98	94.21 ± 1.42	97.88 ± 1.06	0.001
Serine	92.73 ± 1.06	96.01 ± 0.14	93.07 ± 1.06	93.35 ± 2.26	0.063

BSF1 = black soldier fly meal produced in December 2019; BSF2 = black soldier fly meal produced in February 2020; BSF3 = black soldier fly meal produced in May 2020; BSF4 = black soldier fly meal produced in October 2020.

ical deterioration (Kamau *et al.*, 2018), and the moisture content of insect meal may influence its shelf life. However, in the current experiment, the water content levels were beneath the recommended verge, showing their potential stability. The average CP content of the tested meals in this trial was 58.64 % on DM. This value is higher than those reported by Gasco *et al.* (2022), Dumas *et al.* (2018), and Kishawy *et al.* (2022), who reported values equal to 56.4%, 47.1%, and 57.9% on DM, respectively. It has to be underlined that in the case of the CP value reported by Kishawy *et al.* (2022), no information is provided on the N-P conversion factor used. In the current comparison, it is assumed that authors used the one proposed to correct the value for the nitrogen embedded in chitin (Janssen *et al.*, 2017). However, if the conventional 6.25 N-P conversion has been used, the real CP content of the BSF meal used by Kishawy *et al.* (2022) would be much lower, around 52%. The use of the correct N-P conversion factor is recommended, as it prevents overestimation of the protein content, and the formulation of unbalanced diets (Gasco *et al.*, 2023). To properly formulate diets, it has also been suggested to use the true protein content of an ingredient, which is obtained by summing the AA values (Mæhre *et al.*, 2018). When examining the CP values (Table 2) and the sum of the AAs (Table 3) of each BSF meal, some differences emerged. Indeed, the CP values calculated with the N-P conversion factor of

TABLE 6 Digestible essential amino acids content (mg/g DM) of the four black soldier fly meals and rainbow trout requirements (mg/g DM)

	BSF1	BSF2	BSF3	BSF4	RTrq
Arginine	28.93	29.11	24.34	27.80	15
Histidine	17.35	17.90	14.49	17.18	8.0
Isoleucine	24.95	25.68	21.33	24.79	11
Leucine	40.28	39.35	34.28	39.48	15
Methionine	10.58	10.53	8.45	9.77	7.0
Lysine	33.81	33.88	27.68	32.98	24
Valine	34.50	35.53	30.14	35.36	12
Threonine	23.44	23.84	20.72	23.88	11
Phenylalanine	25.61	26.06	21.94	25.00	9.0

BSF1 = black soldier fly meal produced in December 2019; BSF2 = black soldier fly meal produced in February 2020; BSF3 = black soldier fly meal produced in May 2020; BSF4 = black soldier fly meal produced in October 2020; RTrq = rainbow trout requirements.

5.62, consistently resulted in higher values than those obtained by summing the amino acids, with an overestimation of about 1 (BSF2), 10 (BSF1 and BSF4), and 15 (BFS3) %. These discrepancies can be attributed to variation in the effectiveness of the protein extraction due to differences in matrices, or interferences from other chemical substances (Mæhre *et al.*, 2018). It seems that errors in formulations using CP can occur even using the proposed and more appropriate N-P conversion factors (Basto *et al.*, 2020). Therefore, when possible, it is advisable to follow the suggestion of Mæhre *et al.* (2018), recognizing that this may not always feasible due to the instruments required for the amino acid analyses or the costs associated with an external laboratory to perform them. Comparing the EAA profile of the four BSF meals used in this trial to a fish meal obtained by mechanically processing tuna by-products (5-02-023; NRC, 2011), which was the fish meal with the closest CP value (63.44 g/100 g DM) to the BSF meals used, the tuna by-product meal presented a slightly higher total EAAs content (261.72 mg/g DM), with the major elevated values reported for arginine (36.88 mg/g DM), methionine (15.81 mg/g DM), and lysine 45.39 mg/g DM). Similarly, slightly low, or high values were shown by the tuna by-product meal compared to BSF1, BSF2, and BSF4 for histidine (18.82 mg/g DM), isoleucine (26.34 mg/g DM), leucine (40.75 mg/g DM), threonine (24.84 mg/g DM), and phenylalanine (23.12 mg/g DM). Tuna by-product reported lower amounts of valine (29.78 mg/g DM) compared to all BSF meals. The BSF3 globally showed lower values for all EAAs. If the data of this study are compared to a soybean meal solvent extracted (5-04-612; NRC, 2011) containing about 54% of CP (on DM) and a total EAA content of 240.44 mg/g DM, the average level of methionine (10.12 mg/g DM) and of lysine (33.11 mg/g DM) resulted higher in BSF meals than in soybean meal (7.78 and 24.89 mg/g DM, respectively). The average EE of the insect meals tested in this study was 5.3% on DM, indicating a lower lipid content when compared to the BSF meal analysed by Gasco *et al.* (2022), Kishawy *et al.* (2022) and Dumas *et al.* (2018), which reported values of 12%, 11.2% and 20.3% expressed on DM, respectively. Disparities in partially defatted meal yields across these studies may be attributed to variations in larvae rearing techniques (Rozali *et al.*, 2022) and/or processing methods (Hurtado-Ribeira *et al.*, 2023). The chitin content of the four BSF meals was in line with the value (6.8% on DM) reported by Gasco *et al.* (2022) using a defatted BSF meal and the same assessment methodology (Woods *et al.*, 2020). Currently, insect chitin quantification is performed with different methods, leading to results that are not always comparable (Oonincx and Finke, 2021). No differences were observed among BSF meals for nutrients and gross energy digestibility. The DM digestibility values, ranging from 82.66% (BSF1) to 90.10% (BSF4), were higher than those stated for rainbow trout using a defatted BSF meal (68.86%) (Gasco *et al.*, 2022). Furthermore, the values obtained in this study are also higher when compared to DM digestibility values of different proteins of animal origin tested in rainbow trout (Lee *et al.*, 2020). Indeed, the authors found ADC_{DM} values of 66.9% (BSF meal), 52.4% and 67.6% (two types of feather meals), 59.1% and 61.9% (two types of poultry by-products meals), and 51% and 63.5% (two different meat and bone meals) (Lee *et al.*, 2020). Only the ADC_{DM} of the marine-origin protein meals (sardine and menhaden meals) was equal to 86.6% for both meals and aligned with our BSF meals,

but it could be considered lower than what usually reported for these ingredients. The authors argued that, in addition to the unavoidable differences in the environment and genetics of the animals, and differences in ingredient processing methods, the feaces collection methodology might also have had an impact. In fact, Lee *et al.* (2020) used the methodology of manual stripping, which may lead to the expulsion of incompletely digested food combined with sloughed cellular components, thus leading to an underestimation of digestibility. Apparent digestibility values for CP ranged from 84.93% (BSF1) to 91.39% (BSF4). These values compare well with ADC values of other animal by-products, such as meat and bone meal (83-88%) or poultry by-products (83.96%) and were similar to the ADC_{CP} value (89.86%) obtained for rainbow trout by Gasco *et al.* (2022) (CP: 56.4% DM) or by Dumas *et al.* (85%) (CP: 47.09% DM) (Dumas *et al.*, 2018), both assessing partially defatted BSF meals. However, the values of the present study resulted lower when compared to herring fish meal (95%) (NRC, 2011). It is known that protein digestibility is affected by different factors, such as the kind of protein, or the degree to which the bonds between AAs and/or other nutrients of the diets can be hydrolysed to free AA. Moreover, if the (heat)-process applied to obtain the product is not properly conducted, it can also lead to the formation of new indigestible linkages in proteins, lowering their digestibility (NRC, 2011). As far as ADC_{EE} is concerned, values were very high (>96%) in all BSF meals tested, with no significant differences among them. Although the digestibility of lipids is influenced by the saturation level of the fats used, and it is known that BSF larvae are rich in lauric acid (C12:0), which could lead to low lipid digestibility values, the meals herein used had a low lipid content (from 4.35% to 6.44% DM). Therefore, the impact of lauric acid may have been negligible, particularly as the lipid source used in the reference diet was fish oil, which is characterised by a high level of unsaturation and, consequently, has an excellent digestibility (Bélanger *et al.*, 2021). No differences among BSF meals were highlighted also for GE digestibility, resulting globally higher that the ones reported by Gasco *et al.* (2022) (81.86%), Lee *et al.* (2020) (61.8%) for BSF meals and similar to values for menhaden and sardine meals (90.2% and 87.0%, respectively). Despite the BSF1, BSF2, BSF3, and BSF4 meals being produced by the same company using the same processing method, certain statistical trends or differences in terms of nutrient and amino acid digestibility were observed. Generally, all the insect meals tested showed an optimal digestibility in terms of nutrient and amino acids. On the other hand, the variations in the nutrient composition of the meals with respect to the time of the year is undeniable. Specifically, the divergency among the minimum and maximum values was encompassed within the following percentages: 3.6% for DM, 7.8% for CP, 50.5% for EE, and 1.9% for GE. Based on this considerable variability, the standardization of the whole production chain, from substrate to meal, could enhance a stable output in terms of nutrient. Furthermore, nutrient variability in the meal could complicate its utilization, as maintaining a standard formulation for livestock feed may became challenging (Gasco *et al.*, 2023). The BSF4 exhibited the highest ADCs values in terms of nutrients and AAs among meals, followed by BSF2. Since BSF2 meal, which was obtained from larvae fed on a different substrate, was digestible like BSF4, it is possible to state that, in this case, the substrate did not affect the larval digestibility. On the other side, BSF1 showed the lowest digestibility coefficients. Generally, protein utilization decreases when trout are fed on increasing protein levels (Ma *et al.*, 2019), in line with the BSF1 meal composition, characterized by the highest protein percentage. Elevated protein content may reduce the antioxidant capacity of the organism, potentially leading to oxidative damage and, consequently, limiting peptide absorption (Ma *et al.*, 2019). In literature, the effect of BSF meals on fish growth and nutrient digestibility is generally linked to their amino acid profile and/or antinutritional factors (Liland *et al.*, 2021). Regarding the essential amino acid that displayed a statistically significant difference in terms of digestibility (Table 5), the meals showed a variation of 20% for histidine, 16% for valine, and 17% for both isoleucine and leucine. This variability in meal composition may be ruled out as the primary cause of the observed results, as it was comparable to that of the other amino acids, where digestibility remained consistent across meals. Throughout diverse biological functions, histidine is involved in the regulation of gastric secretion, tissue inflammation process, and appetite control (Glover and Wood, 2008). In fish-based diet, histidine is not considered a limited amino acid. However, given the reduction of fish meal in fish diet (applied from 2007-2008 to control feed prices) it is crucial to evaluate histidine availability and digestibility within alternative diets (Hossain *et al.*, 2021). In the present research, the BSF meals were characterized by a large amount of histidine, exceeding the recommended levels (i.e. 0.8%; NRC, 2011), and the digestibility percentages were higher than 90%, comparable to the results obtained by Hossain *et al.* (2021).

In particular, the authors tested different histidine levels through highly digestible free amino acid (Hossain *et al.*, 2021) and, since the histidine digestibility was similar, the results achieved in the current study could be considered as standard. Given their nonlinear and aliphatic side chains, isoleucine, leucine and valine collectively constitute the group of branched-chain amino acids. In rainbow trout, similar to terrestrial animals, an imbalance in branched-chain amino acids can yield antagonistic effects, thus directly impacting nutrient utilization (Yamamoto *et al.*, 2004). Specifically, when fish are fed an excess of leucine, negative effects on isoleucine and valine digestion are observed (Yamamoto *et al.*, 2004). Consequently, it is plausible to hypothesize that in BSF1, leucine may have reduced the availability of the other two essential branched-chain amino acids. Notably, isoleucine plays a crucial role in promoting insulin release, facilitating protein synthesis in muscle tissues, and modulating the secretion of leptin and glucagon-like peptide 1 hormones (Ahmad *et al.*, 2022). These hormonal activities, in turn, contribute to the regulation of blood glucose levels (Ahmad *et al.*, 2022). Conversely, valine participates in tissue repair, maintains nitrogen balance, and contributes to the synthesis of protein and amine neurotransmitters, such as serotonin (Ahmad *et al.*, 2022). Considering leucine, isoleucine and valine, the results obtained in the current trial align with those obtained by Gasco *et al.* (2022), with the exception of the digestibility of the valine in BSF1, which was lower (95 vs 90%), thus supporting the aforementioned theory. While rainbow trout can synthesize the non-essential amino acids, there is no guarantee that the quantity is sufficient to meet the requirements (Hou *et al.*, 2015). Furthermore, the functions of non-essential amino acids are intricately linked to animal metabolism, nutrient digestion, and absorption (Hou *et al.*, 2015). Consequently, assessing their digestibility becomes necessary, as it could potentially enhance animal performance and overall health (Hou *et al.*, 2015). The BSF1 displayed the lowest digestibility for alanine, glycine, and proline. In reference to Gasco *et al.* (2022) findings on BSF meal digestibility, BSF1 exhibited a lower value for alanine, while glycine was reduced in all the tested meals (BSF1, 2, 3 and 4). Conversely, proline digestibility was higher in BSF1, 2, 3, and 4 when compared to Gasco *et al.* (2022) study. Considering that meals produced by the same company determined statistically significant differences, it is possible to hypothesise that the outcomes obtained in the two studies are influenced by both the meal producers (i.e. substrate used, insect strain, processing methods) as well as the fish batch. Finally, the varied digestibility coefficients in BSF1 could be attributed to the storage period that the meal underwent and/or to a process that requires a few finishing touches. Finally, comparing the digestible EAA values of the BSF meals to the requirements of rainbow trout (NRC, 2011) (Table 6), we can see that all the meals largely covered the requirements. Therefore, we can state that these meals are of particular biological value for trout as they are characterised by high EAA bioavailability.

5 Conclusion

Overall, all the insect meals tested exhibited optimal digestibility in terms of nutrients and AAs. Some differences were observed among the batches, and it is well-known that digestibility is closely linked to animal performance. Given the exponential growth in both scientific and entrepreneurial interest in insect rearing over the last decade, significant strides have been made in advancing rearing techniques. In conclusion, aware of the progress within this research sector, the promotion and implementation of standardized production is not negligible, with the aim of meeting market demands.

Conflict of interest

The authors declare that they have no competing interests.

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References

Ahmad, I., Ahmed, I. and Dar, N.A., 2022. Effects of dietary isoleucine on growth performance, enzymatic activities, antioxidant properties and expression of TOR related genes in rainbow trout, *Oncorhynchus mykiss* fingerlings. Aquaculture Research 53: 2366-2382. https://doi.org/10.1111/are.15755

Ajani, E.K., Nwanna, L.C. and Musa, B.O., 2004. Replacement of fishmeal with maggot meal in the diets of Nile tilapia, *Oreochromis niloticus*. World Aquaculture 35: 52-54.

AOAC International, 2000. Official methods of analysis of AOAC International. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

AOAC International, 2003. Official methods of analysis of AOAC International. 17th ed., 2nd revision. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bélanger, A., Sarker, P.K., Bureau, D.P., Chouinard, Y. and Vandenberg, G.W., 2021. Apparent digestibility of macronutrients and fatty acids from microalgae (*Schizochytrium* sp.) fed to rainbow trout (*Oncorhynchus mykiss*): a potential candidate for fish oil substitution. Animals 11: 456. https://doi.org/10.3390/ani11020456

Biasato, I., Chemello, G., Bellezza Oddon, S., Ferrocino, I., Corvaglia, M.R., Caimi, C., Resconi, A., Paul, A., van Spankeren, M., Capucchio, M.T., Colombino, E., Cocolin, L., Gai, F., Schiavone, A. and Gasco, L., 2022. *Hermetia illucens* meal inclusion in low-fishmeal diets for rainbow trout (*Oncorhynchus mykiss*): effects on the growth performance, nutrient digestibility coefficients, selected gut health traits, and health status indices. Animal Feed Science and Technology 290: 115341. https://doi.org/10.1016/j.anifeedsci.2022.115341

Bureau, D.P., Harris, A.M. and Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180: 345-358. https://doi.org/10.1016/S0044-8486(99)00210-0

Caimi, C., Biasato, I., Chemello, G., Bellezza Oddon, S., Lussiana, C., Malfatto, V.M., Capucchio, M.T., Colombino, E., Schiavone, A., Gai, F., Trocino, A., Brugiapaglia, A., Renna, M. and Gasco, L., 2021. Dietary inclusion of a partially defatted black soldier fly (*Hermetia illucens*) larva meal in low fishmeal-based diets for rainbow trout (*Oncorhynchus mykiss*). Journal of Animal Science and Biotechnology 12: 50. https://doi.org/10.1186/s40104-021-00575-1

Choubert, G., De la Noue, J. and Luquet, P., 1982. Digestibility in fish: improved device for the automatic collection of feces. Aquaculture 29: 185-189. https://doi.org/10.1016/0044-8486(82)90048-5

Colombo, S.M., Roy, K., Mraz, J., Wan, A.H.L., Davies, S.J., Tibbetts, S.M., Øverland, M., Francis, D.S., Rocker, M.M., Gasco, L., Spencer, E., Metian, M., Trushenski, J.T. and Turchini, G.M., 2023. Towards achieving circularity and sustainability in feeds for farmed blue foods. Reviews in Aquaculture 15: 1115-1141. https://doi.org/10.1111/raq.12766

Commission Regulation, 2022. Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. Official J. L177/4.

Dumas, A., Raggi, T., Barkhouse, J., Lewis, E. and Weltzien, E., 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 492: 24-34. https://doi.org/10.1016/j.aquaculture.2018.03.038

Espe, M., Andersen, S.M., Holen, E., Rønnestad, I., Veiseth-Kent, E., Zerrahn, J.E. and Aksnes, A., 2014. Methionine deficiency does not increase polyamine turnover through depletion of hepatic S-adenosylmethionine in juvenile Atlantic salmon. British Journal of Nutrition 112: 1274-1285. https://doi.org/10.1017/S0007114514002062

EUMOFA, 2021. Fishmeal and fish oil: Production and trade flows in the EU. European Market Observatory for Fisheries and Aquaculture, Brussels, Belgium.

European Commission, 2017. Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein. Official Journal L138: 92-116.

Gasco, L., Bellezza Oddon, S., Vandenberg, G.W., Veldkamp, T. and Biasato, I., 2023. Factors affecting the decision-making process of using insect-based products in animal feed formulations. Journal of Insects as Food and Feed. https://doi.org/10.3920/JIFF2022.0164

Gasco, L., Caimi, C., Trocino, A., Lussiana, C., Oddon, S.B., Malfatto, V., Anedda, R., Serra, G., Biasato, I., Schiavone, A., Gai, F. and Renna, M., 2022. Digestibility of defatted insect meals for rainbow trout aquafeeds. Journal of Insects as Food and Feed 8: 1385-1399. https://doi.org/10.3920/JIFF2021.0160

Glover, C.N. and Wood, C.M., 2008. Histidine absorption across apical surfaces of freshwater rainbow trout intestine: mechanistic characterization and the influence of copper. Journal of Membrane Biology 221: 87-95. https://doi.org/10.1007/s00232-007-9088-y

Guerreiro, I., Serra, C.R., Coutinho, F., Couto, A., Castro, C., Rangel, F., Peres, H., Pousão-Ferreira, P., Matos, E., Gasco, L., Gai, F., Oliva-Teles, A. and Enes, P., 2021. Digestive enzyme activity and nutrient digestibility in meagre (*Argyrosomus regius*) fed increasing levels of black soldier fly meal (*Hermetia illucens*). Aquaculture Nutrition 27: 1353-5773. https://doi.org/10.1111/anu.13172

Hossain, M.S., Lee, S., Small, B.C. and Hardy, R.W., 2021. Histidine requirement of rainbow trout (*Oncorhynchus mykiss*) fed a low fishmeal-based diet for maximum growth and protein retention. Aquaculture Research 52: 3785-3795. https://doi.org/10.1111/are.15224

Hou, Y., Yin, Y. and Wu, G., 2015. Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. Experimental Biology and Medicine 240: 997-1007. https://doi.org/10.1177/1535370215587913

Hurtado-Ribeira, R., Villanueva-Bermejo, D., García-Risco, M.R., Hernández, M.D., Sánchez-Muros, M.J., Fornari, T., Vázquez, L. and Martin, D., 2023. Evaluation of the interrelated effects of slaughtering, drying, and defatting methods on the composition and properties of black soldier fly (*Hermetia illucens*) larvae fat. Current Research in Food Science 7: 100633. https://doi.org/10.1016/j.crfs.2023.100633

Janssen, R.H., Vincken, J.P., van den Broek, L.A., Fogliano, V. and Lakemond, C.M., 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. Journal of Agricultural and Food Chemistry 65: 2275-2278. https://doi.org/10.1021/acs.jafc.7b00471

Kamau, E., Mutungi, C., Kinyuru, J., Imathiu, S., Tanga, C., Affognon, H., Ekesi, S., Nakimbugwe, D. and Fiaboe, K.K.M., 2018. Moisture adsorption properties and shelf-life estimation of dried and pulverised edible house cricket *Acheta domesticus* (L.) and black soldier fly larvae *Hermetia illucens* (L.). Food Research International 106: 420-427. https://doi.org/10.1016/j.foodres.2018.01.012

Karapanagiotidis, I.T., Neofytou, M.C., Asimaki, A., Daskalopoulou, E., Psofakis, P., Mente, E., Rumbos, C.I. and Athanassiou, C.G., 2023. Fishmeal replacement by full-fat and defatted *Hermetia illucens* prepupae meal in the diet of gilthead seabream (*Sparus aurata*). Sustainability 15: 786. https://doi.org/10.3390/su15010786

Kishawy, A.T., Mohammed, H.A., Zaglool, A.W., Attia, M.S., Hassan, F.A., Roushdy, E.M., Ismail, T.M. and Ibrahim, D., 2022. Partial defatted black solider larvae meal as a promising strategy to replace fish meal protein in diet for Nile tilapia (*Oreochromis niloticus*): performance, expression of protein and fat transporters, and cytokines related genes and economic efficiency. Aquaculture 555: 738195. https://doi.org/10.1016/j.aquaculture.2022.738195

Lee, S., Chowdhury, M.K., Hardy, R.W. and Small, B.C., 2020. Apparent digestibility of protein, amino acids and gross energy in rainbow trout fed various feed ingredients with or without protease. Aquaculture 524: 735270. https://doi.org/10.1016/j.aquaculture.2020.735270

Liland, N.S., Araujo, P., Xu, X.X., Lock, E.J., Radhakrishnan, G., Prabhu, A.J.P. and Belghit, I., 2021. A meta-analysis on the nutritional value of insects in aquafeeds. Journal of Insects as Food and Feed 7: 743-759. https://doi.org/10.3920/JIFF2020.0147

Liland, N.S., Biancarosa, I., Araujo, P., Biemans, D., Bruckner, C.G., Waagbø, R., Torstensen, B.E. and Lock, E.J., 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLOS ONE 12: e0183188. https://doi.org/10.1371/journal.pone.0183188

Ma, R., Liu, X., Meng, Y., Wu, J., Zhang, L., Han, B., Qian, K., Luo, Z., Wei, Y. and Li, C., 2019. Protein nutrition on sub-adult triploid rainbow trout (1): dietary requirement and effect on anti-oxidative capacity, protein digestion and absorption. Aquaculture 507: 428-434. https://doi.org/10.1016/j.aquaculture.2019.03.069

Maehre, H.K., Dalheim, L., Edvinsen, G.K., Elvevoll, E.O. and Jensen, I.J., 2018. Protein determination – method matters. Foods 7: 5. https://doi.org/10.3390/foods7010005

Mohan, K., Rajan, D.K., Muralisankar, T., Ganesan, A.R., Sathishkumar, P. and Revathi, N., 2022. Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: a review of past and future needs. Aquaculture 553: 738095. https://doi.org/10.1016/j.aquaculture.2022.738095

Moutinho, S., Oliva-Teles, A., Martínez-Llorens, S., Monroig, Ó. and Peres, H., 2022. Total fishmeal replacement by defatted *Hermetia illucens* larvae meal in diets for gilthead seabream (*Sparus aurata*) juveniles. Journal of Insects as Food and Feed 8: 1455-1468. https://doi.org/10.3920/JIFF2021.0195

NRC (National Research Council), 2011. Nutrient requirements of fish and shrimp. National Academy Press, Washington, DC, USA.

Ogunji, J., Schulz, C. and Kloas, W., 2008. Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turkish Journal of Fisheries and Aquatic Sciences 8: 141-147.

Oonincx, D.G.A.B. and Finke, M.D., 2021. Nutritional value of insects and ways to manipulate their composition. Journal of Insects as Food and Feed 7: 639-659. https://doi.org/10.3920/JIFF2020.005

Our World in Data, 2023. Soybeans: are they used for food, feed or fuel? World. Available at: https://ourworldindata.org/grapher/soybean-production-and-use

Pauly, D., Zeller, D. and Palomares, M.L.D., 2020. Sea around us concepts, design and data. Available at: https://seaaroundus.org

Radhakrishnan, G., Silva, M.S., Lock, E.J., Belghit, I. and Philip, A.J.P., 2022. Assessing amino acid solubility of black soldier fly larvae meal in Atlantic salmon (*Salmo salar*) in vivo and in vitro. Frontiers in Physiology 13: 2439. https://doi.org/10.3389/fphys.2022.1028992

Ritchie, H., 2021. Is our appetite for soy driving deforestation in the Amazon? Available at: https://ourworldindata.org/soy

Rozali, N.S., Zainuddin, N.A.M. and Mohd, S.R., 2022. Study on the extraction of black soldier fly (*Hermetia illucens*) larvae oil by using mechanical press method. Journal of Sustainability Science and Management 17(12): 49-66. http://doi.org/10.46754/jssm.2022.12.006

van Huis, A., 2020. Insects as food and feed, a new emerging agricultural sector: a review. Journal of Insects as Food and Feed 6: 27-44. https://doi.org/10.3920/JIFF2019.0017

van Huis, A. and Gasco, L., 2023. Insects as feed for livestock production. Science 379: 138-139. https://doi.org/10.1126/science.adc9165

Weththasinghe, P., Hansen, J.Ø., Mydland, L.T. and Øverland, M., 2022. A systematic meta-analysis based review on black soldier fly (*Hermetia illucens*) as a novel protein source for salmonids. Reviews in Aquaculture 14: 938-956. https://doi.org/10.1111/raq.12635

Woods, M.J., Goosen, N.J., Hoffman, L.C. and Pieterse, E., 2020. A simple and rapid protocol for measuring the chitin content of *Hermetia illucens* (L.) (Diptera: Stratiomyidae) larvae. Journal of Insects as Food and Feed 6: 285-290. https://doi.org/10.3920/JIFF2019.0030

Yamamoto, T., Shima, T. and Furuita, H., 2004. Antagonistic effects of branched-chain amino acids induced by excess protein-bound leucine in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 232: 539-550. https://doi.org/10.1016/S0044-8486(03)00543-X