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JOURNAL OF INSECTS AS FOOD AND FEED (2024) DOI:10.1163/23524588-00001425

Journal of Insects as Food and Feed
brill.com/jiff

RESEARCH ARTICLE

Impact of processing technologies on insect meal digestibility in rainbow trout (*Oncorhynchus mykiss*) and European sea bass (*Dicentrarchus labrax*)

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Received 19 October 2024 | Accepted 21 November 2024 | Published online 11 December 2024

Abstract

The objective of this study was to compare the apparent digestibility coefficients (ADCs) of nutrients and energy of three differently processed black soldier fly (BSF) meals in rainbow trout and European sea bass. The processing techniques included defatting with heat treatment followed by tricanter centrifugation, with or without an enzyme hydrolysis step, as well as microwave drying. In the experimental design, each processed BSF meal was mixed with a reference diet at a 30% inclusion level, using celite as an inert digestibility marker and fed to triplicate groups of rainbow trout and European sea bass. For the European sea bass trial, an additional diet with full fat oven-dried BSF meal was also evaluated. In rainbow trout, all the processed insect meals exhibited high digestibility, with no significant differences among the BSF meals. All the amino acids were highly digestible, with ADCs ranging from 84.8% to 93.6% for the essential amino acids and from 78% to 93.2% for the non-essential ones without significant differences between treatments ($P > 0.05$). In European sea bass, while fat and energy digestibility was similar, protein digestibility was significantly higher in all the processed BSF meals when compared to the oven-dried meal ($P = 0.004$). Between the two full fat insect meals, microwave drying significantly improved dry matter and protein digestibility of BSF meal when compared to oven-drying ($P = 0.020$ and $P = 0.004$, respectively). No differences were observed in the digestibility of the two defatted insect meals ($P > 0.05$), thus suggesting that enzymatic hydrolysis did not affect their digestibility in either fish species. This study suggests that processing techniques such as defatting and microwave drying can enhance the nutritional quality and digestibility of BSF meals and offers insights for optimizing insect-based feeds in aquaculture.

Keywords

black soldier fly – defatting – enzymatic hydrolysis – microwave drying – tricanter centrifugation

1 Introduction

As global aquaculture continues to grow to meet the increasing demand for seafood, it is essential to adopt sustainable and efficient feed ingredients. Insect meals present great potential as a sustainable source of protein, offering high nutritional value, ease of production, and minimal environmental impact. Their use provides a promising solution to the increasing demand for nutrients while supporting the sustainability of the aqua-

Published with license by Koninklijke Brill BV | DOI: 10.1163/23524588-00001425
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culture industry. Processing of insect meals has been employed not only to ensure the safety and nutritional quality of the product, but also to improve the techno-functional properties and create functional ingredients for food, feed, pharmaceutical and industrial applications (Liceaga, 2021). Various processing techniques such as drying, blanching, defatting, enzymatic treatments, ultrasonic treatments, protein solubilization and isoelectric precipitation have been developed (Nongonierma and FitzGerald, 2017).

Drying methods are used to lower the total water content in insects, increase shelf-life, and reduce the weight of the insects along with the associated storage and transport costs (Lenaerts *et al.*, 2018; Melgar-Lalanne *et al.*, 2019). Moreover, the removal of water can inhibit microbial growth and spoilage reactions, enzymatic and non-enzymatic activities, browning reactions, and reduce degradation reactions such as lipid oxidation (Kröncke *et al.*, 2018). Oven-drying is commonly used as a traditional drying method; however, it is time and energy consuming. Water evaporates from the surface of the product due to the applied heat, while the remaining moisture slowly diffuses to the surface (Kalla and Devaraju, 2017). Modern techniques, such as freeze-drying/lyophilization are the current commercial methods for stabilizing insects and removing moisture. Due to the low temperatures applied, freeze-drying can remove water, inhibit microbial growth and degradative reactions, and preserve the nutritional quality and heat-sensitive nutrients of the insects (Kröncke *et al.*, 2018; Lenaerts *et al.*, 2018). However, long processing times, along with high capital and operating costs, make lyophilization one of the most expensive drying methods (Parniakov *et al.*, 2022). Microwave drying, on the other hand, offers advantages over conventional heating methods, especially in terms of processing times and energy consumption, making it one of the most promising food processing techniques in various industrial applications (Kalla and Devaraju, 2017). During microwave drying, electromagnetic waves penetrate the material, generating heat within it and drying it volumetrically in a short time (Kalla and Devaraju, 2017). However, similar to oven-drying, microwave drying involves heat, which can lead to nutrient degradation, browning, oxidative damage, protein denaturation and other effects (Lenaerts *et al.*, 2018).

Defatting is a common processing method to remove excess fat from insect meals. Insect larvae have a very high fat content, so this technique can increase protein content, facilitate transportation and storage, and improve the drying and extrusion processes of the meals (Sindermann *et al.*, 2021). Defatting methods include mechanical pressing with a hydraulic press, extraction with organic solvents, supercritical CO_2 extraction, and aqueous extraction (Liceaga, 2021). Organic solvent extractions require pre-dried insect biomass and may not be considered eco-friendly or food-grade due to the use of solvents. Additionally, solvent extraction can lead to protein losses as some proteins may have an affinity to the solvents and can be removed along with them (Nongonierma and FitzGerald, 2017).

Aqueous extraction allows for the simultaneous extraction of fat, soluble protein and insoluble materials, while preserving the nutritional value of lipids and proteins and resulting in food-grade products (Latif and Anwar, 2011). Though this wet fractionation, fat can be utilised as food/feed ingredient or for biodiesel production, while insect protein can be used in food and feed, and chitin can be isolated for the use in food, pharmaceutical, and industrial applications (Caligiani *et al.*, 2018). However, aqueous extraction methods may result in low fat recovery yields due to the formation of oil/water emulsions or the embedding of fat into insoluble aggregates (Azzollini *et al.*, 2020).

Insect fat is stored throughout the body, surrounding the internal organs and the body, between the muscle and the exoskeleton, entangled with protein and chitin (Azzollini *et al.*, 2020; Su *et al.*, 2019). Thus, an effective fat extraction may require the disruption of the insect's structure to facilitate fractionation and release of fat into the medium. Similarly to oil seeds, an enzymatic treatment can be employed to insect meals to degrade the tissue and cell structures, solubilise and hydrolyse the protein matrix, and facilitate fat extraction (Latif and Anwar, 2011; Su *et al.*, 2019). Moreover, enzymatic hydrolysis can aid to the release of cuticular proteins from chitin, which can make them more bio-available, increase protein digestibility, break down allergenic proteins, and result in peptides that present bio-functional activities such as increased antioxidant properties (Batish *et al.*, 2020; Leni *et al.*, 2020b; Liceaga, 2019).

The different processing and extraction methods can result in varying extraction yields, composition, lipid profiles and insect proteins with different functional properties (Ojha *et al.*, 2021; Queiroz *et al.*, 2023). Since proteins are the most important macronutrient for fish nutrition, these variations can affect the nutritional quality of feed. Evaluating a new feed ingredient for the use in aquaculture feeds requires the assessment of its nutritional value for specific fish species by measuring its digestibility and the species' ability to utilize it effec-

tively. Considering the aforementioned pros and cons of different processing methods for insect meals, it is hypothesized that these methods could influence the nutritional quality, and therefore the digestibility of the insect meals. The objective of this study is the evaluation of the effects of different processing methods on the digestibility of black soldier fly in rainbow trout and European sea bass. The processing techniques examined include defatting with heat treatment followed by tricanter centrifugation, with or without an enzyme hydrolysis step, as well as microwave drying.

2 Materials and methods

The study was designed to test the hypothesis that treatment influences digestibility of insect meals in fish using the described methodology. This approach ensures the validity with scientific standards (data for statistical analysis) as well compliance with ethical considerations on the use and handling of experimental animals. The trials were designed and conducted in accordance with the European Directive guidelines (EU 2010/63) on the protection of animals used for scientific purposes. The trial involving rainbow trout was carried out at the experimental facility of the Department of Agricultural, Forest and Food Sciences (DISAFA) of the University of Turin (UNITO, Italy) and the experimental protocol was approved by the UNITO Ethical Committee (protocol No 15731). The trial involving European sea bass took place at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR, Greece) and was authorised by the ethics committee of the region of Crete Greece (License No 255340).

Insect meals and composition of the diets

The black soldier fly meals were produced by Bioflytech S.L. (Murcia, Spain). The experimental insect meals were prepared as follows:

- BSFM (microwaved): the whole larvae were killed by blanching, dried using an industrial-scale, continuous microwave dryer at 22 kW for 15 minutes (MEAM Dry 32, MEAM, Belgium) and subsequently milled at Bioflytech (Vandeweyer *et al.*, 2022).
- BSFH (heated): the whole larvae were killed by freezing and milled using a hand blender to obtain a paste. The paste was treated thermically at 90 °C for four hours, under agitation and acidic conditions using citric acid. The processed paste was centrifuged at Bioflytech using a tricanter. After the centrifugation, the light liquid phase containing the fats was removed, and the heavy liquid phase, containing water and soluble proteins, was concentrated using an evaporator at 60 °C. The concentrated aqueous fraction was then mixed with the solid fraction, dried, and finely milled to produce the insect meal.
- BSFE (enzymatically treated): the whole larvae were killed by freezing, defrosted and milled using a hand blender to obtain a paste. The enzymatic process, conducted at the Leitat Technological Center (Terrassa, Spain), involved the mixture of the paste with 0.2% of a commercial endoprotease, 0.75% of a commercial granulate lipase and 0.75% of a commercial liquid lipase in a bioreactor under acidic conditions (citric acid), in moderate temperatures and agitation for four hours (specific details of the process are not available due to confidentiality). Following enzymatic hydrolysis, the mixture underwent thermal treatment at 90 °C for two hours and tricanter centrifugation at Bioflytech. Similar to BSFH, the heavy liquid phase was concentrated, mixed with the solid fraction, dried, and homogenised to create the insect meal.

The proximate composition and the amino acid profile of the processed BSF meals are shown in Table 1.

To assess the nutritional value and digestibility of the insect meals subjected to the different treatments, the test ingredients were incorporated into a reference diet at a 30% level (70% reference diet:30% test ingredient) following the recommended protocol (Bureau *et al.*, 1999). The reference diet contained 1% acid insoluble ash (Celite® S, Sigma-Aldrich, St. Louis, MO, USA) as an external and inert digestibility marker. The dry ingredients were weighed separately and mixed. Subsequently, fish oil was incorporated into the mixture, followed by the addition of water at a ratio of 50% w/w to achieve a dough-like texture. Pellets were prepared using a meat-mincing machine with an appropriate die and dried at 40 °C for 48 hours. The ingredients and the proximate composition of the experimental diets are presented in Table 2.

Fish and experimental conditions

For rainbow trout, 240 fish, of an average individual weight of 157.7 ± 9.0 g, were obtained from a commercial farm (Troticultura Bassignana, Cuneo, Italy), divided into 12 cylindroconical tanks of 250 L (20 fish per tank, three tanks per diet) equipped with a continuous automatic device to collect the faeces (Choubert *et al.*, 1982). A flow-through open system supplied artesian well water of constant temperature of 13 ± 1 °C.

TABLE 1 Proximate and amino acid composition of the processed and the initial BSF meals expressed in a dry matter basis

	BSFW	BSFH	BSFE	BSFM
Crude Protein (%) [1]	38.0	46.3	46.2	33.9
Crude fat (%)	24.0	15.6	17.6	21.7
Gross energy	22.0	22.3	22.8	23.2
Ash (%)	11.3	10.8	10.7	10.9
Essential amino acids (mg/g)				
Arginine		20.2	17	20.2
Histidine		9.9	8.8	10
Isoleucine		17.7	15.3	17.8
Leucine		29.9	26.6	29.9
Lysine		24.5	20.4	24.5
Methionine		6.9	6.4	6.9
Phenylalanine		17.7	15.5	17.8
Threonine		17.8	15.2	17.8
Valine		25	21.1	25.1
Non-essential amino acids (mg/g)				
Alanine		33	31	33
Aspartic acid		39	35	39
Glutamic acid		50	40	50
Glycine		22.8	19	22.8
Hydroxyproline		<0.6	<0.6	<0.6
Proline		25.3	22.3	25.2
Serine		19.3	16.5	19.2
Taurine		<0.6	<0.6	<0.6
Tyrosine		24	22	24

Abbreviations: BSF = black soldier fly; BSFW = unprocessed full fat, oven-dried, whole BSF larvae meal; BSFM = BSF dried with microwaves; BSFH = BSF processed with heating treatment and tricanter centrifugation; BSFE = BSF treated enzymatically and tricanter centrifugation.

1 Using a nitrogen to protein conversion factor of 4.67 for the full fat BSFs (BSFM and BSFW) and 5.60 for the defatted ones (BSFH and BSFE) (Janssen *et al.*, 2017).

After a 14-day acclimatization with the diets, during which faeces were not collected, the fish were fed by hand to visual satiety twice daily, seven days a week, for four weeks. Faeces were collected from each automatic device twice per day (at 8:00 and 15:00), pooled, frozen, freeze-dried and stored at –20°C until subsequent analysis.

For European sea bass, 120 fish, of an average individual weight of 189.0 ± 25.9 g, were supplied from the IMBBC and divided into 15 cylindroconical tanks of 250 L equipped with a settling column (8 fish per tank, three tanks per diet). A flow-through open system supplied borehole water of constant temperature of 20 ± 0.2 °C. For the adaptation period, the fish were fed by hand three times a day until apparent satiation for 10 days, without collecting the faeces, to acclimate them to the experimental diets. Throughout the three-week collection phase, the fish were hand-fed until apparent satiation three times a day, seven days a week. Any unconsumed feed was removed, and the settling columns were adjusted to the tanks until the following morning. Prior to the morning feeding, the faeces were collected, centrifuged at 2000 g for 10 min, then pooled per tank and stored in –20 °C. At the conclusion of the trial the faeces were freeze-dried and stored at –20 °C until analysis.

Proximate and chemical analysis of diets and faeces

For the rainbow trout trial, all the analyses were performed at the DISAFA facilities, whereas for the European sea bass trial, they were conducted at the IMBBC facilities. Dry matter and ash were analysed according to AOAC (2000) using the methods #934.01 and #942.05, respectively. Crude protein was determined using the method #984.13 (AOAC, 2000) at DISAFA and a nitrogen analyser at IMBBC (FP-528, Leco corporation, St. Joseph, MI, USA), employing a nitrogen-to-protein conversion factor of 6.25 for the diets, 5.60 for

TABLE 2 Ingredients and proximate composition (dry matter basis) of the experimental diets

	Reference diet	BSFM	BSFH	BSFE	BSFW
Ingredients (gr/kg wet weight)					
Fish meal	500	350	350	350	350
Wheat gluten	20	14	14	14	14
Soybean meal	165	115.5	115.5	115.5	115.5
Wheat meal	120	84	84	84	84
Starch gelatinized	80	56	56	56	56
Celite®	10	7	7	7	7
Vitamins and minerals Premix	5	3.5	3.5	3.5	3.5
Fish oil	100	70	70	70	70
Test ingredient	–	300	300	300	300
Proximate composition for the rainbow trout trial					
Protein (%)	47.4	47.8	48.4	48.1	
Fat (%)	14.1	14.3	13.4	13.4	
Energy (MJ/kg)	19.1	20.3	18.6	18.5	
Ash (%)	10.8	11.5	10.9	10.7	
Proximate composition for the European sea bass trial					
Protein (%)	53.9	49.9	52.3	52.8	49.3
Fat (%)	19.4	21.5	20.1	20.2	21.8
Energy (MJ/kg)	23.7	23.7	23.6	23.7	23.6
Ash (%)	8.6	9.4	9.3	9.3	10.4

Abbreviations: BSF = black soldier fly meal; BSFM = BSF dried with microwaves; BSFH = BSF processed with heating treatment and tricanter centrifugation; BSFE = BSF treated enzymatically and tricanter centrifugation; BSFW = unprocessed full fat, oven-dried, whole BSF larvae meal.

the defatted insect meals and 4.67 for the full fat insect meals (Janssen *et al.*, 2017). Fat content was assessed using either the ether extract method #2003.05 at DISAFA (AOAC, 2003) or the chloroform/methanol extraction method at IMBBC (Folch *et al.*, 1957). The gross energy content was determined using adiabatic bomb calorimeters (C7000, IKA, Staufen, Germany at DISAFA and 6300, Parr Instrument Company, St. Moline, IL, USA at HCMR). Celite content in diets and faeces was determined with the acid insoluble ash method for both facilities (Vogtmann *et al.*, 1975). The amino acid composition of the diets and faeces of the rainbow trout trial was determined by a high-resolution liquid chromatography (HPLC, Agilent 1200, Agilent Technologies Inc., Santa Clara, CA, USA). Samples were hydrolysed with 6 N HCl solution and 4 M NaOH for 22 h at 110 °C in a nitrogen atmosphere. The digested samples were evaporated in a rotor evaporator and re-suspended in 50 ml of borate buffer. The supernatant was diluted in 0.1 N HCl by adding an internal sarcosine standard (SAR). Amino acids were derivatized with OPA / Fmoc and a binary gradient. The solvents used to make the gradient of elution were A = 100% Acetate buffer in 18 min and B = 60% H_2O ultrapure / methanol / acetonitrile (20/40/40 v/v/v) in 16 min. A solid column of 250 × 4 mm in internal diameter (Hypersil BDS-C18 5 μm) was maintained at 18°C. The eluted amino acids were quantified with a Diode Array detector.

Calculation of the apparent digestibility coefficients and statistical analysis

Apparent digestibility coefficients (ADCs) of nutrients and energy of the reference and experimental diets were determined using the acid insoluble ash (AIA) as a measurement of the external digestibility marker and the following formula:

$$\text{Apparent Digestibility Coefficient (ADC)} = 100 - 100 \times (\text{AIA in the diet/AIA in faeces}) \times (\text{nutrient or energy in faeces /nutrient or energy in the diet}).$$

The ADC of dry matter (DM) was calculated as follows:

$$ADC_{DM} = 100 - 100 \times (\text{AIA in the diet/AIA in faeces}).$$

The ADCs of the nutrients and energy of the individual tested ingredients were determined using the following formula:

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

where ADC_{test} = the ADC (%) of a nutrient or energy of the experimental diet, ADC_{ref} = the ADC (%) of a nutrient or energy of the reference diet, D_{ref} = the % of nutrient or MJ/kg of energy in the reference diet expressed in a dry matter basis, D_{ing} = the % of nutrient or MJ/kg of energy of the test ingredient expressed in a dry matter basis.

The ADC_{DM} of the ingredients were calculated using the formula:

$$ADC_{ing} = 100/30 \times (ADC_{test} - 0.7 \times ADC_{ref})$$

where ADC_{ing} = the dry matter ADC of the test ingredient, ADC_{test} = the dry matter ADC of the test diet and ADC_{ref} = the dry matter ADC of the reference diet.

Data were analyzed by one-way analysis of variance (ANOVA) to determine if significant differences existed among the dietary treatments (n = 3 tanks per diet; results were considered statistically significant at $P < 0.05$), while individual means were compared using the post-hoc Tukey's test. The Kolmogorov-Smirnov and Levene's tests were used to test normality and equality of variances of the data, respectively. Statistical analyses were carried out using SigmaStat 3.5 (Systat Software, Inc., San Jose, CA, USA) and IMB SPSS Statistics v. 27.0 (IBM, Armonk, NY, USA). The results are expressed as the mean and pooled standard error of the mean (SEM).

3 Results

The nutrient, energy and amino acid ADCs of the processed BSF meals in rainbow trout are presented in Table 3. The digestibility of the different BSF meals was very high, with no statistically significant differences among them. All the amino acids were highly digestible, with ADCs ranging from 84.8% to 93.6% for the essential amino acids and from 78% to 93.2% for the non-essential ones, without significant differences between treatments ($P > 0.05$).

Table 4 shows the nutrient and energy ADCs of insect meals for the European sea bass trial. In European sea bass, all the processed insect meals demonstrated significantly higher protein digestibility (81.9-86.5%) when compared to the oven-dried insect meal (67.8%; $P = 0.004$). Fat digestibility was similar among all the experimental meals (78.1-82.6%; $P > 0.05$). The one-way ANOVA revealed a statistically significant difference in energy ADC ($P = 0.040$), however post-hoc analyses did not identify any statistically significant differences between any specific pairs.

4 Discussion

This study evaluated the impact of different processing techniques on the digestibility of black soldier fly meals in rainbow trout and European sea bass. The study involved a full fat insect BSF meal processed using a novel dielectric drying method (microwave drying) and two freeze-dried, defatted insect meals, which underwent a heating treatment followed by tricanter centrifugation with or without an enzymatic hydrolysis step. For the sea bass trial an additional full fat insect meal produced using standard industrial production methods (oven-drying) was tested.

In rainbow trout, all the processed insect meals were highly digestible, with no statistically significant differences among them. Insect meals have been studied extensively in the diets of rainbow trout with very positive outcomes. Rema *et al.* (2019) using graded levels of partially defatted yellow mealworm (*Tenebrio molitor*, TM) meal in an inclusion level up to 25% to completely replace fish meal, observed no differences in diet digestibility and an overall improvement of growth performance and feed utilization in small rainbow trout juveniles (initial weight 5 g initial weight) as inclusion levels increased. In larger rainbow trout (initial weight 78 g), the inclusion of up to 20% defatted TM for the complete replacement of fish meal did not have any influence on growth performance, though higher inclusion levels resulted in decreased protein digestibility (Chemello *et al.*, 2020). Despite the reduced dry matter and fat digestibility in diets including 50% full fat TM, protein digestibility was higher compared to the control diet, resulting in improved growth performance (Belforti *et al.*, 2015). In the present trial, the ADCs of crude protein and essential amino acids for all BSF meals were high and similar to the mechanically defatted BSF meal used in the study of Dumas *et al.* (2018) on rainbow trout. However, the high chitin content (2.73%) in the BSF used by Dumas *et al.* may have contributed to lower ADC_{EE} and ADC_{DM} values (43% and 69%, respectively), as the authors suggested.

TABLE 3 Apparent digestibility coefficients (%) of processed black soldier fly (BSF) meals in rainbow trout

	BSFH	BSFE	BSFM	SEM	p-value
Dry matter	75.0	76.7	72.3	1.66	0.62
Crude protein	84.2	83.8	83.8	0.49	0.94
Ether extract	98.4	98.0	97.5	0.35	0.62
Gross energy	78.5	78.6	83.4	1.38	0.28
Essential amino acids					
Arginine	90.5	93.6	87.3	2.38	0.62
Histidine	89.9	90.8	86.1	1.87	0.61
Isoleucine	89	92.2	87.5	1.28	0.74
Leucine	88.4	91.9	87.3	2.23	0.74
Lysine	88.5	91.5	87.6	2.65	0.86
Methionine	88.9	92	84.8	3.03	0.69
Phenylalanine	89.3	93	85.7	2.18	0.46
Threonine	88.4	90.9	85.5	2.05	0.62
Valine	89.2	91	86.6	1.98	0.72
Non-essential amino acids					
Alanine	89.9	91.3	89.2	1.6	0.89
Aspartic acid	86.6	89.8	86.3	2.41	0.84
Glutamic acid	87.5	90.4	85.1	2.79	0.78
Glycine	85.9	85.8	78	2.2	0.27
Proline	88.8	89.3	85.8	1.73	0.73
Serine	87.8	90	84.3	2.17	0.63
Tyrosine	92.6	93.2	89.2	1.46	0.56

Abbreviations. BSFH = defatted and heat treated BSF meal; BSFE = defatted, heat and enzymatically treated BSF meal; BSFM = full fat and microwave dried BSF meal. The results are expressed as the mean and pooled standard error of the mean (SEM), n = 3 tanks per diet.

TABLE 4 Apparent digestibility coefficients (%) of processed back soldier fly (BSF) meals in European sea bass

	BSFH	BSFE	BSFM	BSFW	SEM	p-value
Dry matter	68.1 [a]	59.9 [ab]	64.2 [a]	36.0 [b]	4.55	0.020
Crude protein	86.5 [a]	83.7 [a]	81.9 [a]	67.8 [b]	1.81	0.004
Crude fat	82.6	78.1	79.9	79.1	2.49	0.62
Gross energy	76.2	71.4	75.8	57.8	2.88	0.040

Abbreviations: BSFH = defatted and heat treated BSF meal; BSFE = defatted, heat and enzymatically treated BSF meal; BSFM = full fat and microwave dried BSF meal; BSFW = full fat, oven-dried, whole BSF larvae meal. Within rows, different letters denote statistically significant difference ($P < 0.05$). The results are expressed as the mean and pooled standard error of the mean (SEM), n = 3 tanks per diet.

In contrast, the partially defatted BSF meals with higher chitin content (6.8-8.56%) used in studies by Gasco *et al.* (2022) and Bellezza Oddon *et al.* (2024) were well utilised by rainbow trout, showing slightly higher ADC_{CP} (89.86% and 84.68-91.39%, respectively) and essential amino acid ADCs (93-100% and 90.0-99.1%, respectively) when compared to the present study. Additionally, the fat in the BSF of these studies was efficiently absorbed, resulting to ADC_{EE} values of 96.25-100%, similar to those observed in the present study (Bellezza Oddon *et al.*, 2024; Gasco *et al.*, 2022). The negative effects of chitin on the nutrient digestibility of many fish species are well-documented (Belghit *et al.*, 2018; Caimi *et al.*, 2020; Fabrikov *et al.*, 2020; Guerreiro *et al.*, 2020). However, the BSF meals used in the rainbow trout studies, also varied in other nutrient components, such as the ash content (12.7% in Dumas *et al.*, 2018, 10.3% in Gasco *et al.*, 2022 and 8.3-10.7% in Bellezza Oddon *et al.*, 2024), which has also been

shown to affect nutrient digestibility (Basto *et al.*, 2020; Mastoraki *et al.*, 2022).

In European sea bass, fat and energy digestibility was similar among the BSF meals. However, the processed insect meals had significantly higher ADC_{CP} compared to the oven-dried BSF (BSFW). Insect meals are generally very well accepted by European sea bass at moderate levels of inclusion in the diets, but their digestibility and overall effects on fish growth can be affected by the presence of indigestible components such as chitin and ash. Research has shown that incorporating TM, BSF or common housefly (*Musca domestica*) meals at levels up to 25% did not negatively impact growth performance or nutrient digestibility (Abdel-Tawwab *et al.*, 2020; Gasco *et al.*, 2016; Magalhães *et al.*, 2017; Mastoraki *et al.*, 2020; Mastoraki *et al.*, 2022). Furthermore, the inclusion of 25% TM or 19.5% of superworm (*Zophobas morio*) even enhanced protein digestibility (Gasco *et al.*, 2016; Mastoraki *et al.*, 2022). However, as the inclusion of TM or BSF increased to over 30%, growth performance was negatively affected (Gasco *et al.*, 2016; Reyes *et al.*, 2020). The decreased ADCs of diets incorporating BSF, lesser mealworm (*Alphitobius diaperinus*) or locust meal were attributed to the high content of indigestible fiber, chitin and ash of the insect meals (Basto *et al.*, 2020; Mastoraki *et al.*, 2022). In the present study, the nutrient and energy ADCs of the BSF meals were lower than the ones reported by Basto *et al.* (2020). However, Basto *et al.* (2020) used a different experimental diet (80% reference diet with 20% BSF meal inclusion), smaller fish (initial weight of 33 g compared to 189 g of the current study) and higher water temperature (22 °C compared to 20 °C in this study), which may have influenced the results.

For the European sea bass trial, between the full fat insect meals, the use of the microwave dried one resulted in higher diet dry matter and protein digestibility, as well as higher ingredient protein and energy digestibility when compared to the oven-dried meal. Microwave drying offers several advantages over oven-drying. Unlike oven-drying, which requires heating the entire atmosphere of the oven and relies on transfer of heat from the surface to the centre of the material, microwave drying involves electromagnetic waves that directly penetrate the materials and generate heat throughout the material. This results in thorough heating and evaporation of water from the inside-out, reducing the risk of overheating of the outer layer (Kalla and Devaraju, 2017; Lenaerts *et al.*, 2018; Puligundla *et al.*, 2013). This inside-out energy transfer leads to shorter drying times, generally lower drying temperatures, reduced energy consumption, and more uniform drying with minimal differences in temperature throughout the layers of the material. Additionally, the rapid drying process, shortens the time that the materials are exposed to higher temperatures, which helps preserve heat-sensitive nutrients such as vitamins B and C, antioxidants, carotenoids and phenols (Kalla and Devaraju, 2017). However, Huang *et al.* (2019) compared the *in vitro* protein digestibility of microwave-dried and oven-dried BSF and reported that high-temperature microwave drying can induce structural changes in protein, such as polymerization. These changes can affect particle size and surface morphology, consequently reducing the accessibility of digestive enzymes and lowering digestibility. In this study, the microwave treatment appears to facilitate the digestion of the full fat insect meal in European sea bass when compared to oven drying.

Regarding all the processed insect meals, no significant differences were observed in their digestibility, in either rainbow trout or sea bass. It was expected that the defatting process would result in more pronounced differences in the nutrient digestibility of the insect meals. However, defatting did not substantially alter the fat content among the processed insect meals and therefore it didn't have any significant effect on digestibility among them. Contrary to BSFM, BSFH and BSFE were lyophilised, without undergoing a blanching pre-treatment. Blanching, apart from being used as a killing method, is also utilised to minimise microbial load and reduce or prevent the endogenous enzymatic activity which can cause browning or spoilage (Parniakov *et al.*, 2022). Enzymatic browning can negatively affect techno-functional properties of proteins and reduce solubility and digestibility (Janssen *et al.*, 2019). The most common blanching methods are boiling water immersion and steam blanching. However, hot water immersion is often associated with nutrient degradation, due to the prolonged exposure to higher temperatures, and soluble nutrient leaching into the surrounding medium (Azzollini *et al.*, 2016; Melgar-Lalanne *et al.*, 2019). In this experiment, the full fat insect meals were steam blanched and, therefore, no differences in nutrient composition or integrity due to boiling were expected.

On the other hand, lyophilization is a drying process which removes moisture from frozen materials through sublimation under vacuum. The absence of heat and oxygen during the dehydration process maintains the nutritional quality of the materials along with their texture, shape, colour, flavour, aroma and biological

activity (Lenaerts *et al.*, 2018; Liceaga, 2021). Lenaerts *et al.* (2018) examined the differences in proximate composition and nutritional quality of freeze-dried and microwave dried TM and they didn't observe any significant differences in the proximate composition, regardless of whether a blanching step was performed. Furthermore, freeze-drying preserved the vitamin B_{12} content of TM when compared to microwave drying, which resulted in lower B_{12} content (Lenaerts *et al.*, 2018). On the other hand, freeze-drying has been found to influence fatty acid composition and quality, increasing unsaturated fatty acid content and oxidation status, and reducing the fat particle size (Lenaerts *et al.*, 2018; Purschke *et al.*, 2018). However, in this study, any potential changes did not influence fat and energy digestibility among the insect meals in either of the fish species studied.

Regarding the two defatted insect meals, no differences in the apparent digestibility coefficients were observed in either fish species studied. The only difference between these meals was the inclusion of an enzymatic hydrolysis step. Enzymatic hydrolysis has been shown to improve the functional properties of the insect meals through the modification of the protein structure (Lamsal *et al.*, 2019). When compared to intact proteins, protein hydrolysates are considered more digestible and easily absorbed due to their smaller peptide size, the modification of the tertiary structure of proteins, the exposure of digestive enzymes' action sites, and changes in interactions with other dietary components (Ajomiwe *et al.*, 2024; Drulyte and Orlien, 2019; Leni *et al.*, 2020b). Additionally, protein hydrolysis has been shown to affect the techno-functional properties of insect proteins such as foaming, emulsifying and gelling properties, as well as protein solubility (Mishyna *et al.*, 2021). Protein solubility is important not only in insect protein applications in food products, but also higher solubility could result in improved digestibility (Davalos-Vazquez *et al.*, 2024). Yoon *et al.* (2019) found that protein solubility of TM increased by 128% after hydrolysis with a mix of commercial proteases (Flavourzyme and Alcalase). Similarly, Alcalase treatment significantly increased the protein solubility of the cricket *Gryllodes sigillatus* to up to 92% at pH 10 (Hall *et al.*, 2017) and a treatment with a commercial protease from *Bacillus licheniformis* increased protein solubility of lesser mealworm by 12-34% (Leni *et al.*, 2020a). In this study, the enzymatic hydrolysis step was utilised as a way to facilitate the defatting process and the advantages that may occur from the production of protein hydrolysates did not affect the nutrient and energy digestibility in either fish species.

5 Conclusions

In rainbow trout, all the processed insect meals exhibited high digestibility, with no significant differences among the various processing methods. However, in European sea bass, while the fat and energy digestibility were similar among the processed meals and the full fat oven-dried BSF meal, differences appeared in the protein and dry matter digestibility due to the processing techniques. Microwave drying showed an advantage over conventional oven-drying, as did defatting, regardless of whether it involved an enzymatic hydrolysis step. Overall, all the processing techniques improved digestibility compared to the conventional full fat oven-dried BSF meal, suggesting that processing can enhance the nutritional quality of BSF meal for European sea bass.

Conflict of interest

The authors have no conflict of interest to declare.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement n° 861976 (Susinchain). This document reflects only the authors' views and the Commission is not responsible for any use that may be made of the information it contains.

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