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Seaweed species and pre-treatment methods: Effects on fatty acid profile and performance in black soldier fly (*Hermetia illucens*) larvae

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ABSTRACT

This study investigated how the species and pre-treatment methods of seaweed incorporated in substrates affects the fatty acid profile and performance of black soldier fly larvae (BSFL; *Hermetia illucens*). Two feeding experiments were conducted. In Experiment 1, 5-day-old BSFL were reared 14 days on eight poultry manure-based substrates: a control (100 % poultry manure), four substrates containing 12 % of either fish offal or a seaweed species (*Kappaphycus alvarezii*, *Gracilaria salicornia*, or *Sargassum wightii*), and three substrates containing 6 % fish offal combined with 6 % of each seaweed species. In Experiment 2, 6-day-old BSFL were reared 14 days on eight poultry manure-based substrates: one with 100 % poultry manure, one with 67 % untreated *K. alvarezii*, and six with 67 % *K. alvarezii* subjected to different pre-treatments: enzyme treatment (Allzyme® at 1.5 % and 2 % w/w), fermentation (10 % and 15 % v/v *Saccharomyces cerevisiae*), or microwave treatment (800 W for 2 and 3 min). Experiment 1 showed that feeding seaweed together with fish offal enhanced the omega-3 content in BSFL ($p < 0.05$). Among the tested seaweeds species, only *K. alvarezii* increased omega-3 levels when used alone, though this came at the cost of reduced larval performance ($p < 0.05$). Experiment 2 demonstrated that pre-treatment of *K. alvarezii* enhanced larval omega-3 fatty acids, compared to untreated seaweed ($p < 0.05$). While both untreated and pre-treated *K. alvarezii* reduced larval performance ($p < 0.05$), pre-treated seaweed showed numerical improvements in performance compared to untreated seaweed. In conclusion, BSFL can bioaccumulate omega-3 from seaweed, with pre-treatment enhancing enrichment efficiency and potentially improving larval performance.

1. Introduction

The use of insects as a sustainable feed source has attracted increasing attention in recent years, driven by their nutrient-rich composition, high feed conversion efficiency, and sustainable production (Ewald et al., 2020). Among insect species, black soldier fly (BSF; *Hermetia illucens*) has garnered significant interest for mass rearing (Siddiqui et al., 2022). The larvae of the BSF (BSFL) are highly efficient scavengers, capable of breaking down a diverse range of organic materials, including manure, decomposing matter,

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lignocellulosic substrates, and both pre- and post-consumer food waste, provided the material is not excessively rigid (Mariod, 2020). The mid-gut of BSFL adapts to diets with varying nutrient content, enabling them to efficiently utilize a wide range of feeding substrates (Bonelli et al., 2020). The BSFL are highly nutritious, containing 31–59 % protein, 11–49 % lipid (Weththasinghe et al., 2021), amino acid profile comparable to fishmeal (Swinscoe et al., 2019), and bioactive compounds such as chitin, antimicrobial peptides, and lauric acid (Li et al., 2022). Nevertheless, BSFL is a poor source of unsaturated fatty acids (Truzzi et al., 2020), particularly, omega 3 fatty acids (Weththasinghe et al., 2021).

Omega-3 fatty acids, especially eicosapentaenoic acid (C20:5, EPA) and docosahexaenoic acid (C22:6, DHA), are crucial for optimal growth and immunity in animals (Alagawany et al., 2019; Kalakuntla et al., 2017; Tocher, 2015). Besides, these fatty acids are known to provide health benefits for humans upon consumption of animal products, due to their preventive role in the onset and progression of multiple health conditions, including cardiovascular diseases (Alfio et al., 2021). High levels of omega-3 fatty acids in animal feed are essential for enriching animal-derived products, thereby enhancing their nutritional and health benefits for human consumption (Kalakuntla et al., 2017). Being a scarce source of omega-3 fatty acids, the dietary inclusion of BSFL decrease the omega-3 fatty acid content in animal products (St-Hilaire et al., 2007a, 2007b). Hence, these limit the usefulness of BSFL as a feed source, potentially creating challenges for both producers and consumers of animal-derived products (El-Dakar et al., 2020).

The nutritional composition of BSFL, particularly their fat content, can change according to the type of rearing substrate (Schacky et al., 2007). Notably, BSFL have been shown to bioaccumulate polyunsaturated fatty acids from their substrate (El-Dakar et al., 2020), further enhancing their value as a sustainable feed ingredient (Giannetto et al., 2020). Several studies have reported that BSFL can be enriched with omega-3 fatty acids by providing substrates comprising omega-3 fatty acids (Liland et al., 2017; St-Hilaire et al., 2007a, 2007b), including seaweed (marine macroalgae) and fish offal (El-Dakar et al., 2020; Erbland et al., 2020; Rodrigues et al., 2022; Vaz et al., 2016). For instance, BSFL were enriched with omega-3 fatty acids when fed on cow manure supplemented with fish offal (St-Hilaire et al., 2007a, 2007b), laying hen feed supplemented with fishmeal (Barroso et al., 2017), linseed oil (Lozica et al., 2020) and processed wheat supplemented with the seaweed *Ascophyllum nodosum* (Liland et al., 2017). The high moisture, salt, and complex carbohydrate content of seaweed makes it less suitable as a direct feed ingredient for many animals (Makkar et al., 2016). Instead, it can be useful in enriching omega-3 fatty acids in BSFL, enabling the larvae to function as carriers of essential nutrients from sources otherwise unsuitable for direct inclusion in animal diets.

The lipid content and fatty acid profile of seaweeds differ both qualitatively and quantitatively depending on genetic factors and environmental conditions, such as sunlight, temperature, and nutrient availability (Jayasinghe et al., 2018). While BSFL are known to accumulate omega-3 fatty acids from seaweed, the comparative effects of different seaweed species on this process remains unexplored. In this context, the present study was designed in two phases. The first experiment evaluated the potential of three locally available seaweed species; *Kappaphycus alvarezii*, *Gracilaria salicornia*, and *Sargassum wightii* alone and in combination with the fish offal to enhance the omega-3 fatty acid content in BSFL. The experiment, aimed to identify the most effective seaweed species for fatty acid enrichment while also evaluating larval growth performance and nutrient conversion efficiency. In Sri Lanka, these seaweed species are primarily collected from the wild, particularly along the southern, eastern, and northern coastal belts, while *K. alvarezii* is also increasingly cultivated in small-scale community-based farming systems. However, there is no local consumption of *K. alvarezii* as it is produced exclusively for exportation. Moreover, a significant portion of the seaweed biomass, particularly from *Sargassum* and *Gracilaria*, remains underutilize. Seasonal blooms of *Sargassum* often result in large volumes washing ashore, posing disposal challenges to coastal communities. Potentially several hundreds of tons of these seaweeds annually can be sustainably valorized as a feed substrate for BSFL (Munisamy et al., 2024), providing an environmentally friendly strategy for both waste management and the enhancement of the nutritional profile of insect biomass.

Previous studies have reported that feeding higher level of seaweed (>50 %) can impair the growth of BSFL due to the complex seaweed structure (Liland et al., 2017). The negative impact on larval growth with seaweed inclusion in substrates was also evident in our Experiment 1. Various pre-treatment methods such as alkaline treatment, acid treatment, autoclaving, microwave treatment, ultrasound, and biological processes like enzymatic hydrolysis and fermentation have been explored to improve the nutrition quality and bioavailability of seaweed by disrupting the complex cell wall matrix and enhancing the release of functional compounds (Aarthy et al., 2018; Campbell et al., 2020; Fernandes et al., 2022). Accordingly, Experiment 2, employed a substantially higher inclusion level (67 %) of pretreated *K. alvarezii* to assess whether processing methods could mitigate the growth limitations typically associated with high seaweed inclusion while enhancing omega-3 fatty acid accumulation in BSFL.

2. Materials and methods

2.1. Experiment 1

2.1.1. Preparation of substrates

Poultry manure were obtained from 6 months old layer chicken operation at the Livestock Field Station, Faculty of Agriculture, University of Peradeniya, Sri Lanka. Yellowfin tuna (*Thunnus albacares*) fish offal were bought from the Municipal Central Market, Kandy, Sri Lanka. The three seaweed species, *K. alvarezii* (9°37'43.22"N, 79°53'1.09"E), *S. wighitti* (9°35'51.58"N, 79°58'53.51"E) and *G. salicornia* (9°35'51.58"N, 79°58'53.51"E) were collected from Jaffna, Sri Lanka. An identification guide was used to identify the seaweed specimens (Coppejans et al., 2011). The specimens were deposited at the Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka for future references. Table S1 contains detailed information and voucher numbers of the seaweed, and their photographs and collected locations are presented in Figure S1. In practice, local producers predominantly use poultry manure as a rearing substrate due to its availability and low cost. Therefore, poultry manure was selected as the control in the

present study to reflect current local practices and ensure the relevance of our findings to on-ground production systems. Fish offal, poultry manure and seaweeds were dried at 60℃ until reaching a constant weight, and ground to powder. The proximate composition of the ingredients are shown in Table S2. Eight substrates were prepared using poultry manure as basal ingredient; PM: 100 % poultry manure; FO: poultry manure supplemented with 12 % of fish offal, KA: poultry manure supplemented with 12 % of *K. alvarezii*; GS: poultry manure supplemented with 12 % of *G. salicornia*; SW: poultry manure supplemented with 12 % of *S. wighitti*; FO+KA: poultry manure supplemented with 6 % of *K. alvarezii* and 6 % of fish offal; FO+GS: poultry manure supplemented with 6 % of *G. salicornia* and 6 % of fish offal and FO+SW: poultry manure supplemented with 6 % of *S. wighitti* and 6 % fish offal. The substrates were moistened with distilled water until a 70 % final moisture level was reached (Makkar et al., 2014), and stored at −20°C until further use.

2.1.2. Experimental set-up

Five days old BSFL were obtained from a local insect producer in Sri Lanka. Until day five, the BSFL have been fed with potato pulp. After arrival, the larvae were hand counted and divided into 24 plastic containers (40 cm × 30 cm ×7.5 cm; 150 larvae per container, 0.125 larvae/ cm^2) covered with a paper with fine holes and sealed. Initial weights of larvae per container were measured (~ 6.10 mg per larva). The larvae were fed with one of the eight substrates for 14 days (n = 3 containers). The substrates were added to the containers once in two days at the rate of 100 mg/larvae/day. During the experimental period, the average room temperature was 26℃ and relative humidity was 88 %. At the end of the 14 days of experiment, larvae were separated from the substrate reside using a sieve and washed with water and dried on paper towels. The larvae were counted, and total weight of larvae and frass per container were recorded. The larvae were frozen at −20℃ for slaughtering, and dried at 60℃ until reaching a constant weight. Dried larvae were ground and stored in air tight bags at 4℃ until further analysis. The proximate and fatty acids compositions of BSFL were measured at the beginning and end of the experiment.

2.2. Experiment 2

2.2.1. Ingredients in substrates

Poultry manure was obtained from 6 months old layer chicken operation at the Livestock Field Station, Faculty of Agriculture, University of Peradeniya, Sri Lanka. The *K. alvarezii* were collected from Jaffna (9°37'43.22"N, 79°53'1.09"E), Sri Lanka. *K. alvarezii* was selected for further studying due to its omega-3 accumulation potential among tested seaweed species in experiment 1. Fish offal was excluded in this experiment since it is usually processed into fishmeal rather than using directly for insect feed. Poultry manure and seaweeds were dried at 60℃ until reaching a constant weight, and ground to powder. The proximate composition of the seaweeds and poultry manure are shown in Table S3.

2.2.2. Pretreatment of seaweeds

2.2.2.1. Enzyme treatment. The seaweed powder was treated with Allzyme® enzyme cocktail at the rates of 1.5 % or 2 % (w/w) on a dry matter basis. Allzyme® contain protease, phytase, cellulose, beta glucanase, amylase and xylanase enzymes. The procedure explained by Matshogo et al.(2021) was followed with a slight modification for the preparation of enzyme treatments. Enzyme solutions were prepared by dissolving 3.3 g or 4.4 g of Allzyme® in 2200 mL of distilled water, which was then sprayed onto 220 g of ground seaweed powder (2 mm particle size) to achieve a 1:10 ratio of seaweed to enzyme solution. The volume of distilled water needed to dissolve the enzyme was estimated using an iterative process, aiming to prevent the leaching of chemical components from the seaweed. Treated seaweeds were stored at room temperature (average 30℃) for 24 h to provide sufficient time for Allzyme® to predigest fiber in seaweeds. Seaweeds were then oven dried at 60℃ until a constant weight was reached, and ground with a mixer grinder (Philips, India).

2.2.2.2. Fermentation. The fermentation treatments were prepared according to Hardjani et al. (2017). Dry yeast (*Saccharomyces cerevisiae*; Mauripan® instant dry yeast) was first activated. Approximately 0.5 g of dry yeast, 5 mL of distilled water and 0.5 g sugar were added into test tube and kept 10–15 min at 38–43℃ in water bath for the activation. Activated dry yeast was cultured in Potato Dextrose Agar (PDA). One colony was isolated and gram strained for identifying the *S. cerevisiae*. A loop of *S. cerevisiae* was inoculated on PDA medium and incubated at 28–30℃ for 24–48 h in incubator (Memmert, DT-120–699416). The strain was activated by transferring 1–2 loops of culture into 100 mL of activation medium and incubating at room temperature (28–30°C) for 24 h. The activation medium consisted of 20 g/L peptone, 10 g/L yeast extract, and 20 g/L glucose. The activation step was repeated by inoculating 10 % (v/v) of culture from the previous step. After activation, *S. cerevisiae* cells were enumerated using a hemocytometer (Marienfeld, Germany) until a density of 10^6 cells/mL was achieved.

The fermentation process was carried out by adding the inoculum to a medium consisting of a 1:10 ratio of seaweed powder to deionized water. The fermentation treatment was done at two levels, i.e., of 10 % (v/v) inoculum and 15 % (v/v) inoculum. The incubation was performed at 28–30℃, under room temperature conditions while stirring once per hour for 72 h. A drying procedure was carried out in a conventional electric oven at 60℃ for 72 h after the completion of the fermentation process. Once dried, seaweed was ground using a mixture grinder (Philips, India).

2.2.2.3. Microwave treatment. Seaweed powder was mixed with distilled water (solid: liquid ratio of 1:10 w/v) and placed in the microwave oven (model MS2387U, LG) at 800 W. The treatment was applied interruptions each 30 s, and two exposure durations were

tested: 2 min and 3 min (Fernandes et al., 2022). Treated seaweed was dried in conventional electric oven at 60°C for 72 h after the completion of the treatment. Once dried, treatment was ground using a mixture grinder (Philips, India).

2.2.3. Substrate preparation

Eight substrates for the BSFL larvae were prepared by mixing different combination of the poultry manure and untreated or pre-treated *K. alvarezii*; PM: 100 % of poultry manure; U-KA: poultry manure + 67 % of untreated *K. alvarezii*; E1.5: poultry manure + 67 % of *K. alvarezii* treated with 1.5 % enzyme; E2: poultry manure + 67 % of *K. alvarezii* treated with 2 % enzyme; F10: poultry manure + 67 % of *K. alvarezii* fermented with 10 % yeast inoculum; F15: poultry manure + 67 % of *K. alvarezii* fermented with 15 % yeast inoculum; M2: poultry manure + 67 % of *K. alvarezii* microwaved for 2 min; M3: poultry manure + 67 % of *K. alvarezii* microwaved for 3 min. Substrates consisted of 70 % of moisture (Makkar et al., 2014). The substrates were stored at −20℃ until further use.

2.2.4. Experimental set-up

Six days old BSFL were obtained from a local insect producer in Sri Lanka. Until day six, the BSFL have been fed with potato pulp. After arrival, the larvae were hand counted and divided into 24 plastic containers (40 cm × 30 cm ×7.5 cm; 400 larvae per container, 0.33 larvae/ cm^2) covered with a paper with fine holes and sealed. Initial weight of larvae per container was weighed (~ 4.2 mg per larva). The increase in larval population size from 100 to 400 per container between experiments was made to better simulate commercial rearing densities, ensuring the applicability of the findings to practical production settings. The larvae were fed with one of the eight substrates for 14 days (n = 3 containers). The substrates were added to the containers once in two days at the rate of 125 mg/ larvae/day. During the experimental period, the average room temperature was 26℃ and relative humidity was 88 %. Upon completion of the 14 days of experiment, larvae were sieved to separate them from the substrate residue, washed with water and dried on paper towels. Total weight of larvae and frass per container were recorded. The larvae were frozen at −20℃ for slaughtering, and dried at 60℃ until reaching a constant weight. Dried larvae were ground and stored in air tight bags at −20℃ until further analysis. The proximate and fatty acids compositions of substrates and BSFL were measured at the beginning and end of the experiment.

2.3. Proximate analysis

The substrates, larvae and frass samples were initially dried at 60℃ to remove surface moisture and then oven dried at 104℃ until reaching a constant weight to determine dry mater content. Ash content was estimated by combustion at 550℃. The samples dried at 60℃ were used for the determination of crude protein, fat and fiber content. The crude protein content was estimated by Kjeldahl method according to the AOAC (1980). Nitrogen conversion factor was 6.25 for all the samples except seaweed and BSFL. The factor of 4.97 was used for the seaweed (Angell et al., 2016), while 4.76 was used for BSFL (Truzzi et al., 2020). Crude fat content was estimated by soxhlet method (Thiex et al., 2003). Crude fiber content was determined by gravimetrically (AOAC, 1980).

2.4. Analysis of fatty acid profile

Initially, 1 g of substrate and BSFL powder was subjected to crude oil extraction using 40 mL of hexane in a Soxhlet extraction system as described by Thiex et al. (2003). The extracted crude oil was then recovered through rotary evaporation under vacuum conditions at 40℃, and weighed.

Fatty acid methyl esters (FAMEs) were identified by comparing their retention time with those of a standard mixture (Supelco® 37 Component FAME Mix, CRM47885, Sigma-Aldrich, USA) and FAME was prepared by thoroughly mixing 60 mg of the oil sample with 0.3 mL of dichloromethane and 2 mL of 0.5 M sodium methoxide in a 15 mL screw-cap methylation tube. The mixture was heated in a water bath at 50°C for 30 min, and then left to cool to room temperature. After cooling, 5 mL of distilled water was added drop by drop, followed by 0.1 mL of glacial acetic acid and 0.5 mL of hexane, with thorough mixing at each step. The solution was allowed to stand at room temperature for 30 min, then the top hexane layer was separated and placed into a 2 mL Gas Chromatography (GC) vial. The vials were sealed with Parafilm™ and kept at −20°C until analysis.

The FAME analysis was performed using a GC system (US 16,443,037, USA) equipped with an Agilent J&W CP-Sil 88 column for FAME (100 m, 250 μm, 0.2 μm). The GC conditions were as follows: injection volume: 1 μL; carrier gas: hydrogen in constant pressure mode; inlet temperature: 260°C, and split ratio: 50:1. The oven program was set to 100°C (held for 5 min), increased by 8 °C/min to 180°C (held for 9 min), and then by 1 °C/min to 230°C (held for 15 min). The flame ionization detector (FID) was maintained at 260°C with airflow settings: hydrogen at 40 mL/min, air at 400 mL/min, and makeup gas at 25 mL/min (Wickramasinghe et al., 2023).

2.5. Larval performance parameters

The performance parameters of larvae were estimated as follows.

Weight gain = Final average body weight - Initial average body weight

Bioconversion efficiency = ((Final larval biomass in dry matter basis – Initial larval biomass in dry matter basis)/ Weight of substrate provided in dry matter basis) × 100 %

Feed conversion ratio (FCR) = Weight of substrate provided in dry matter basis/ (Final larval biomass in dry matter basis – Initial larval biomass in dry matter basis)

Waste reduction = ((Weight of substrate provided in dry matter basis – Weight of frass at harvest in dry matter basis)/ Weight of substrate provided in dry matter basis) × 100 %

Nitrogen conversion efficiency = (Final larval nitrogen content – Initial larval nitrogen content)/ (Weight of substrate provided × Nitrogen content in substrate /100) × 100 %

2.6. Statistical analysis

In both experiments, data were analyzed separately using one-way analysis of variance (ANOVA) (n = 3), followed by Duncan's post-hoc test for mean comparisons, performed using IBM SPSS Statistics 26. The level of significance was set at $p < 0.05$. One-way ANOVA was considered appropriate because, in each experiment, treatments were designed as independent groups differing by a single experimental factor. In both experiments, treatments varied in substrate composition, allowing comparison of the effect of substrate types on BSFL performance and composition. Prior to ANOVA, the data were tested for homogeneity of variance by Levene's test and normal distribution of residution of residuals using Kolmogorov-Smirnov test. Correlations between nutrient contents and performance parameters of the substrates and BSFL were assessed using Pearson correlation tests and visualized as correlation matrices in GraphPad Prism version 9.3.1.

In both experiments, poultry manure was used as the basal diet; however, its composition differed due to batch variation, which is common with organic substrates. In addition, differences in the starting age and initial weight of the BSFL, as well as potential variations in environmental conditions, may have contributed to the performance differences observed between the control groups. To avoid complications arising from these differences, each experiment was analyzed and interpreted using its own respective control diet, ensuring valid comparisons within, rather than across, experiments.

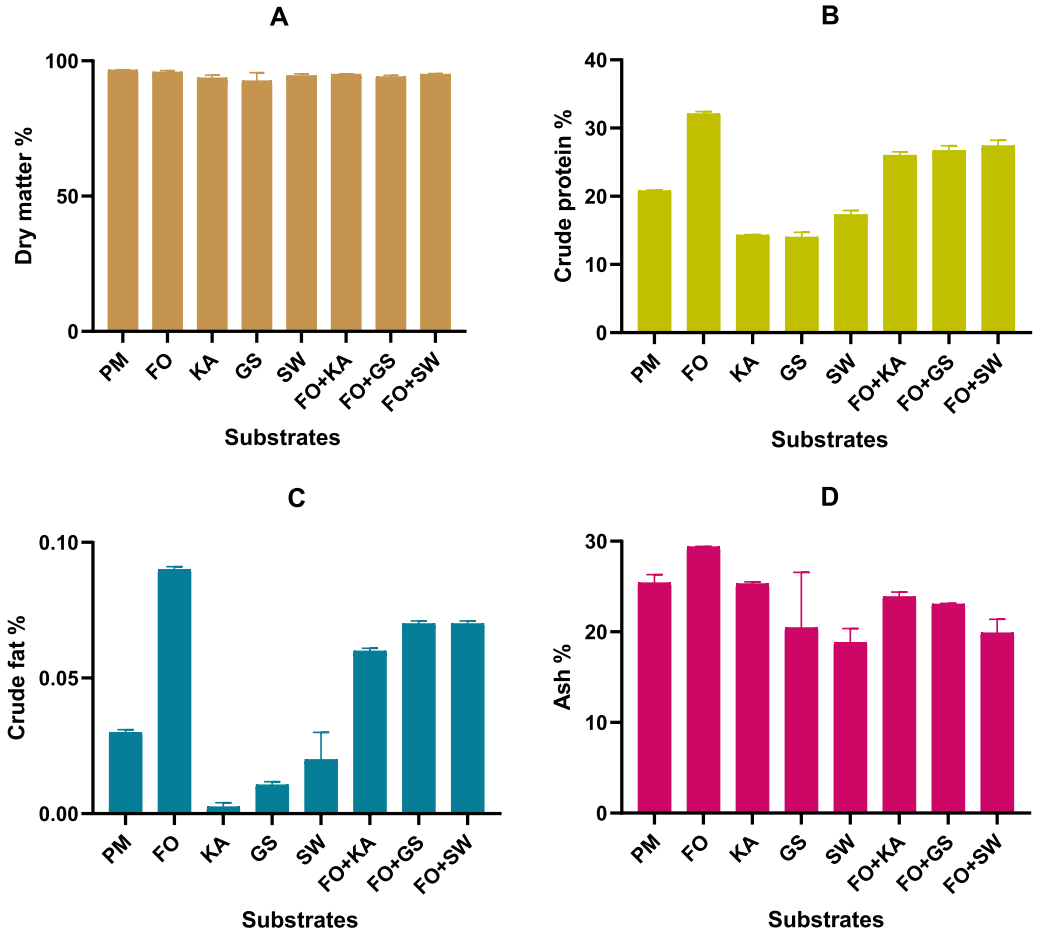

Fig. 1. Proximate composition (% dry matter) of eight substrates fed to black soldier fly larvae in Experiment 1. A: dry matter (%); B: crude protein; C: crude fat; D: ash; PM: poultry manure (100 %); FO: fish offal; KA: *Kappaphycus alvarezii*; GS: *Gracilaria salicornia*; SW: *Sargassum wightii*; FO+KA: fish offal + *K. alvarezii*; FO+GS: fish offal + *G. salicornia*; FO+SW: fish offal + *S. wightii*.

3. Results

3.1. Experiment 1

3.1.1. Larval nutrient and fatty acid composition

The proximate compositions of the substrates and BSFL post-feeding are presented in Figs. 1 and 2, respectively. The inclusion of fish offal increased the protein and fat contents of the substrates, whereas the inclusion of seaweeds reduced these nutrient levels. Larval protein content was similar across 100 % poultry manure and most other substrates; however, the inclusion of *K. alvarezii* and *S. wightii* with fish offal led to a reduction ($p < 0.05$) in protein levels compared to 100 % poultry manure. Among the dietary treatments, larvae fed with the substrate containing 12 % fish offal exhibited the highest ($p < 0.05$) crude fat content. Other substrates yielded similar fat contents in the larvae, comparable to those fed only poultry manure. The inclusion of 12 % fish offal resulted in the lowest ($p < 0.05$) ash content in the BSFL. Substrates supplemented solely with seaweed increased ($p < 0.05$) the ash content in the larvae, whereas the combination of seaweed and fish offal led to a reduction ($p < 0.05$). No correlations were observed between substrate and larval protein and fat contents (Fig. 3).

Among the eight substrates tested, only five contained detectable levels of EPA and DHA. These include the substrate containing 12 % fish offal, three substrates containing both fish offal and seaweed, and the substrate supplemented with 12 % *K. alvarezii*. No omega-3 fatty acids were detected in the substrate composed of 100 % poultry manure or in the substrates supplemented only *G. salicornia* or *S. wightii* (Table 1). The fatty acid profiles of the larvae fed eight substrates are shown in Table 2. Initially, the BSFL mainly contained lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1cis (n9)), and linoleic acid (C18:2cis (n6)). At the end of the feeding period, EPA and DHA were not detected in larvae fed exclusively on poultry manure. In contrast, these fatty acids were present in larvae reared on substrates containing fish offal, regardless of whether seaweeds were included. The larvae fed the substrate containing 12 % *K. alvarezii* also exhibited detectable levels of EPA and DHA. However,

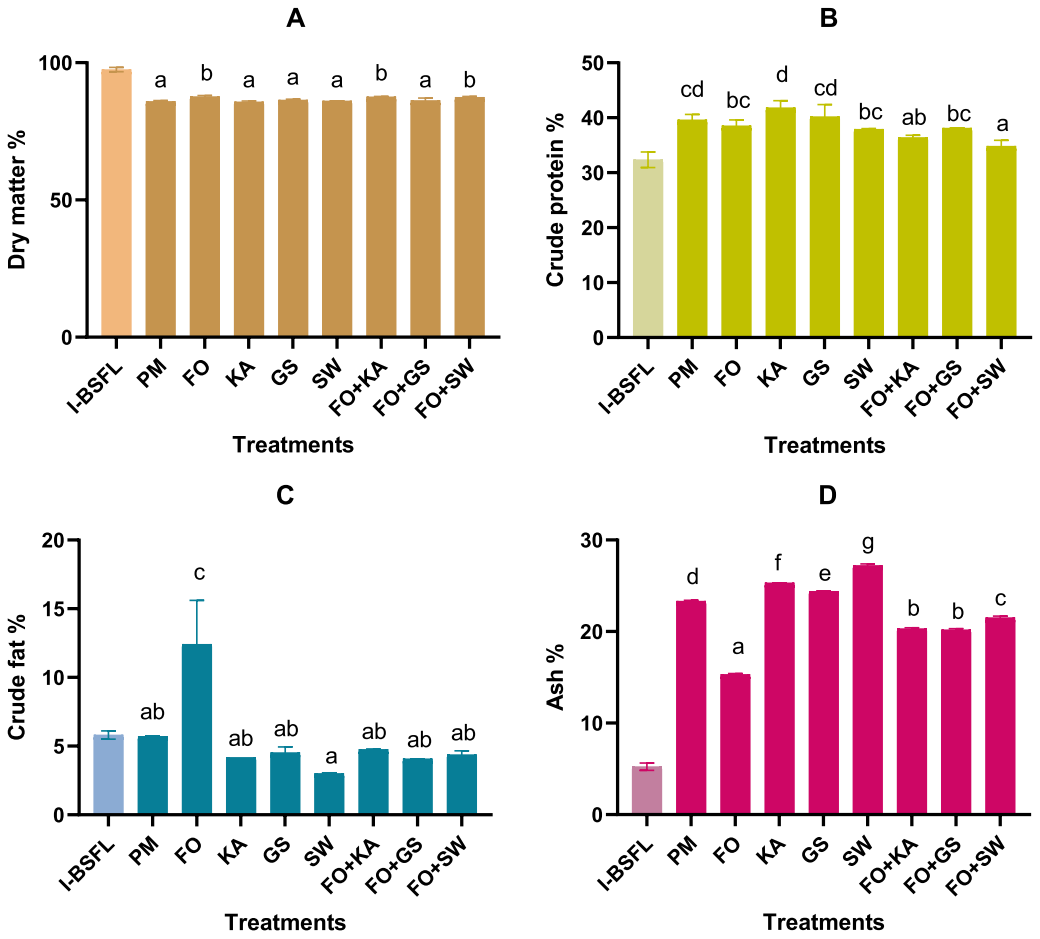

Fig. 2. Proximate composition (% dry matter) of black soldier fly (*Hermetia illucens*) larvae fed with eight substrates in Experiment 1. A: dry matter (%); B: crude protein; C: crude fat; D: ash; I-BSFL: initial black solder fly larvae; PM: poultry manure (100 %); FO: fish offal; KA: *Kappaphycus alvarezii*; GS: *Gracilaria salicornia*; SW: *Sargassum wightii*; FO+KA: fish offal + *K. alvarezii*; FO+GS: fish offal + *G. salicornia*; FO+SW: fish offal + *S. wightii*. Bars with different letters within each graph are significantly different ($p < 0.05$) according to Duncan multiple range test.

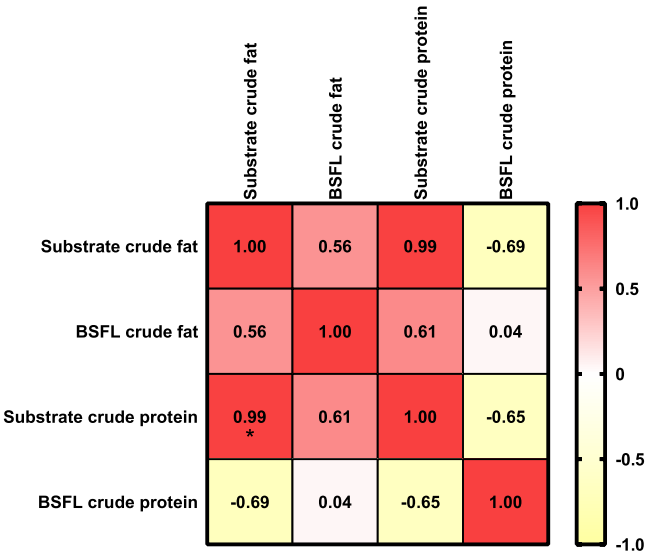

Fig. 3. Correlation matrix of protein and fat contents between black soldier fly larvae (BSFL) and their substrates in Experiment 1. The Pearson correlation coefficients are shown numerically, with asterisks indicating significance levels (*$p < 0.05$).

Table 1
Fatty acid composition (% of total fatty acids) of eight substrates fed to black soldier fly larvae in Experiment 1.

Fatty acid	PM	FO	KA	GS	SW	FO + KA	FO + GS	FO + SW
C12:0	1.02 ± 0.0	ND	1.12 ± 0.0	1.05 ± 0.0	2.27 ± 0.0	1.06 ± 0.0	0.33 ± 0.0	0.58 ± 0.0
C14:0	1.18 ± 0.0	3.60 ± 0.4	2.95 ± 0.0	1.76 ± 0.0	4.89 ± 0.0	2.73 ± 0.0	2.86 ± 0.0	3.39 ± 0.0
C16:0	23.8 ± 0.4	21.2 ± 0.3	26.7 ± 0.0	28.1 ± 0.3	26.5 ± 0.1	23.4 ± 0.7	22.2 ± 0.7	25.6 ± 0.2
C16:1	ND	5.33 ± 0.0	2.01 ± 0.0	ND	ND	3.16 ± 0.0	3.88 ± 0.0	3.87 ± 0.0
C18:0	11.5 ± 0.2	7.79 ± 0.0	12.7 ± 0.3	16.1 ± 0.3	14.7 ± 0.2	7.22 ± 0.0	8.46 ± 0.0	8.45 ± 0.0
C18:1cis(n9)	31.5 ± 0.3	16.4 ± 0.2	16.5 ± 0.1	15.7 ± 0.5	20.9 ± 0.5	20.06 ± 0.0	20.77 ± 0.0	21.15 ± 0.0
C18:2 cis (n6)	21.7 ± 0.3	3.69 ± 0.0	10.9 ± 5	23.9 ± 0.7	28.0 ± 0.1	9.48 ± 0.0	6.00 ± 0.1	2.76 ± 0.0
C20:5n3 (EPA)	ND	18.80 ± 0.4	16.3 ± 0.0	ND	ND	26.7 ± 0.2	15.6 ± 0.2	16.0 ± 0.0
C22:6n6 (DHA)	ND	22.9 ± 0.5	7.73 ± 0.0	ND	ND	9.01 ± 0.0	10.0 ± 0.0	8.55 ± 0.0

Values are expressed as mean ± standard deviation. PM: poultry manure (100 %); FO: fish offal; KA: *Kappaphycus alvarezii*; GS: *Gracilaria salicornia*; SW: *Sargassum wightii*; FO+KA: fish offal + *K. alvarezii*; FO+GS: fish offal + *G. salicornia*; FO+SW: fish offal + *S. wightii*; ND: not detected; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

substrates supplemented solely with *S. wightii* or *G. salicornia*, in the absence of fish offal, did not result in EPA or DHA enrichment in the larvae. Larvae fed 100 % poultry manure exhibited the highest levels of capric acid (C10:0) ($p < 0.05$). Moreover, this group showed higher ($p < 0.05$) concentrations of lauric acid (C12:0) and myristic acid (C14:0) compared to larvae fed the substrate supplemented with 12 % fish offal (Table 2). Correlation analyses indicated positive relationships ($p < 0.05$) in DHA, EPA and omega 6 linolenic acid contents between substrates and larvae (Fig. 4).

3.1.2. Larval performance and nutrient utilization

The performance indicators of BSFL fed eight different substrates are shown in Table 3. Larvae fed the substrate containing 12 % fish offal recorded the highest final body weight ($p < 0.05$), while the inclusion of 12 % *K. alvarezii* or *G. salicornia* reduced ($p < 0.05$) final body weight. No significant reduction in final body weight was observed in larvae fed substates supplemented *S. wightii* alone or combinations of fish offal and seaweed. Larvae fed 12 % fish offal exhibited the lowest FCR ($p < 0.05$), whereas supplementation with 12 % seaweed led to higher ($p < 0.05$) FCR values. FCR of larvae fed substrates containing both fish offal and seaweed were comparable to those fed 100 % poultry manure. Substrates with 12 % seaweed reduced ($p < 0.05$) waste reduction efficiency, while fish offal, whether fed alone or in combination with seaweed, did not negatively affect this parameter. The highest bioconversion efficiency was observed in larvae fed 12 % fish offal, followed by those receiving both fish offal and seaweed. The larvae fed 12 % seaweed exhibited the lowest bioconversion efficiency. In contrast, nitrogen conversion efficiency was higher ($p < 0.05$) in larvae fed substrates containing 12 % *K.alvarezii* or *G. salicornia*, whereas it was lower ($p < 0.05$) in larvae fed fish offal, regardless of seaweed inclusion. The survival rate of BSFL was significantly affected by the dietary treatments. The highest ($p < 0.05$) survival rate was observed in larvae fed 12 % fish offal, while the lowest survival rate ($p < 0.05$) was recorded in larvae fed 12 % *S. wightii*. Additionally, positive correlations ($p < 0.05$) were observed between larval EPA levels and larval waste reduction, as well as between larval DHA levels and larval final body weight, weight gain, and bioconversion efficiency (Figure S2).

Table 2
Fatty acid composition (% of total fatty acids) of black soldier fly larvae fed eight substrates in Experiment 1.

Fatty acid	I-BSFL	PM	FO	KA	GS	SW	FO+ KA	FO+ GS	FO+ SW	p value
C10:0	ND	1.05 ± 0.7^{b}	0.42 ± 0.7^{a}	< 0.001^{a}	< 0.001^{a}	< 0.001^{a}	< 0.001^{a}	< 0.001^{a}	< 0.001^{a}	0.01
C12:0	8.21 ± 0.4	33.6 ± 3.9^{c}	13.1 ± 6.7^{a}	29.5 ± 7.7^{bc}	21.8 ± 14.8^{abc}	17.3 ± 6.2^{ab}	10.9 ± 0.3^{a}	10.7 ± 0.3^{a}	11.3 ± 0.2^{a}	0.01
C14:0	3.36 ± 0.2	8.42 ± 2.2^{b}	5.9 ± 0.4^{a}	7.16 ± 1.9^{ab}	6.52 ± 0.1^{ab}	6.38 ± 0.3^{a}	5.74 ± 0.4^{a}	6.28 ± 0.2^{a}	6.53 ± 0.1^{ab}	0.13
C16:0	19.2 ± 0.7	20.8 ± 3.9^{ab}	24.5 ± 1.4^{bc}	18.0 ± 0.02^{a}	21.3 ± 5.2^{ab}	23.8 ± 0.3^{b}	29.2 ± 4.1 ^{cd}	30.5 ± 0.1^{d}	32.1 ± 0.6^{d}	< 0.001
C16:1	ND	4.11 ± 0.1^{a}	5.04 ± 1.6^{ab}	5.65 ± 0.4^{ab}	7.93 ± 3.7^{ab}	8.61 ± 1.0^{b}	7.03 ± 2.3^{ab}	5.84 ± 0.4^{ab}	6.65 ± 2.4^{ab}	0.18
C18:0	7.49 ± 0.9	3.46 ± 2.1^{ab}	4.55 ± 4.2^{abc}	5.59 ± 0.3^{bc}	1.72 ± 0.04^{a}	9.42 ± 0.1^{d}	6.58 ± 0.6^{bcd}	7.43 ± 1.9 ^{cd}	6.94 ± 0.01 ^{cd}	0.18
C18:1cis (n9)	28.2 ± 1.2	9.23 ± 2.8	17.3 ± 7.1	9.63 ± 3.5	11.0 ± 4.2	9.18 ± 4.2	12.9 ± 5.4	9.69 ± 5.1	8.37 ± 3.4	0.71
C18:2 cis (n6)	30.6 ± 1.5	3.39 ± 1.1^{ab}	0.67 ± 0.2^{a}	4.07 ± 4.2^{ab}	3.27 ± 2.2^{ab}	5.69 ± 1.0^{b}	2.02 ± 1.5^{ab}	1.92 ± 1.2^{ab}	2.14 ± 1.9^{ab}	0.31
C20:5n3 (EPA)	ND	< 0.0001^{a}	5.07 ± 0.0^{bc}	1.47 ± 3.3^{ab}	< 0.001^{a}	< 0.001^{a}	4.70 ± 3.3^{bc}	6.00 ± 0.9^{c}	1.44 ± 2.0^{ab}	0.01
C22:6n3 (DHA)	ND	< 0.0001^{a}	2.99 ± 1.3^{c}	0.57 ± 0.1^{ab}	< 0.001^{a}	< 0.001^{a}	1.77 ± 0.9^{bc}	2.12 ± 1.8^{bc}	0.80 ± 0.3^{ab}	0.00

Values are expressed as mean ± standard deviation. I-BSFL: initial black solider fly larvae; PM: poultry manure (100 %); FO: fish offal; KA: *Kappaphycus alvarezii*; GS: *Gracilaria salicornia*; SW: *Sargassum wightii*; FO+KA: Fish offal + *K. alvarezii*; FO+GS: Fish offal + *G. salicornia*; FO+SW: Fish offal + *S. wightii*; ND: not detected; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Values in the same row with different superscript letters are significantly different ($p < 0.05$) according to Duncan multiple range test. p value: p value for one way ANOVA.

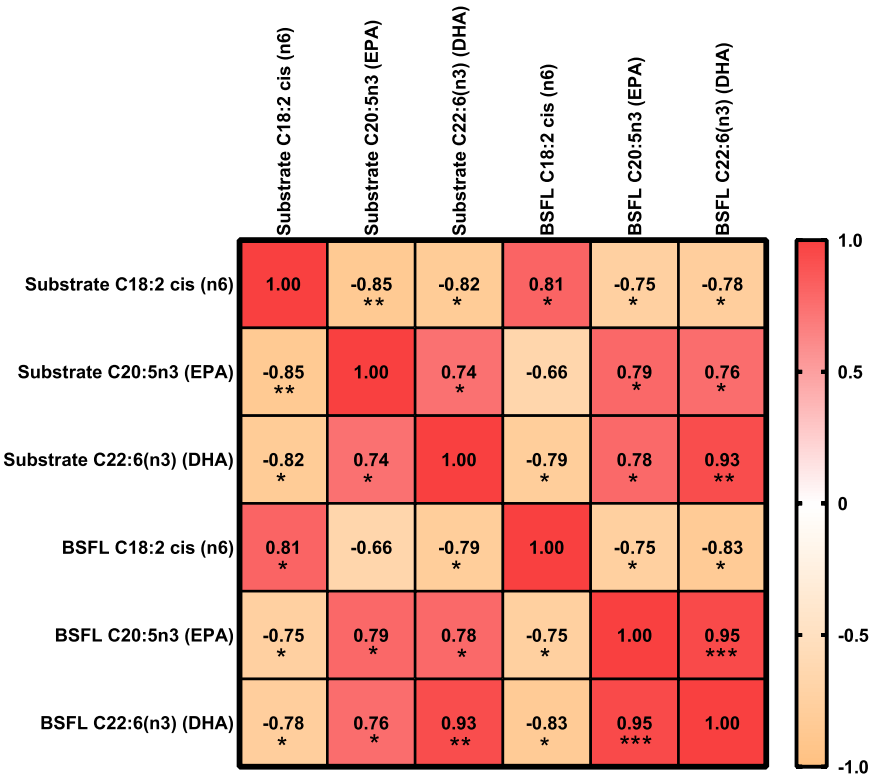

Fig. 4. Correlation matrix between the fatty acid contents (% of total fatty acids) of black soldier fly larvae (BSFL) and the corresponding fatty acid contents (% of total fatty acids) in their substrates in Experiment 1. The Pearson correlation coefficients are shown numerically, with asterisks indicating significance levels (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). n6: omega 6 fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

3.2. Experiment 2

3.2.1. Larval nutrient and fatty acid composition

Crude protein, fiber, and ash contents were lower in substrates supplemented with seaweed compared to the 100 % poultry manure substrate. The protein contents were comparable among the substrates containing untreated and treated seaweed. Substrates containing microwaved seaweed showed numerically higher fat content. In general, the inclusion of pre-treated seaweed increased the fat and fiber contents compared to untreated seaweed, with the exception of the substrate containing 1.5 % enzyme-treated seaweed, which did not follow this trend (Fig. 5).

The proximate compositions of BSFL fed eight different substrates are shown in Fig. 6. Larvae fed pre-treated seaweed generally had comparable protein content as those fed no or untreated seaweed, except larvae fed seaweed fermented at a 10 % (v/v) rate, whom exhibited higher ($p < 0.05$) protein content. Fat content was higher ($p < 0.05$) in larvae fed microwaved seaweed for 2 min and enzyme-treated seaweed at a 1.5 %, than larvae fed only poultry manure. Ash content was also elevated ($p < 0.05$) in larvae fed fermented or microwaved seaweed compared to those receiving substrates without seaweed or with untreated seaweed. No significant correlations were observed in protein and fat contents between substrates and larvae (Fig. 7).

Omega-3 fatty acids were detected in all seven substrates containing seaweed. However, substrates with pre-treated seaweed generally showed lower omega-3 content compared to the substrate with untreated seaweed, except for the one containing 1.5 % enzyme-treated seaweed, which exhibited higher DHA content (Table 4). As observed in Experiment 1, the initial BSFL contained lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 cis (n9)), and linoleic acid (C18:2 cis (n6)). By the end of the feeding period, omega-3 fatty acids were present in larvae fed both untreated and pre-treated seaweed. Notably, larvae fed 15 % fermented or 2 min microwaved seaweed exhibited higher total omega-3 content than those fed untreated seaweed. Compared to the untreated seaweed fed group, EPA content was higher in larvae fed pre-treated seaweed substrates, except those treated with 2 % enzyme or microwaved for 3 min. In contrast, DHA content was lower in larvae fed all pre-treated seaweed substrates compared to those fed untreated seaweed (Table 5).

Increasing the rate of enzyme treatment of seaweed from 1.5 % (w/w) to 2 % (w/w), as well as extending microwave exposure at 800 W from 2 min to 3 min, reduced ($p < 0.05$) the total omega-3 fatty acid content in BSFL. Conversely, increasing the fermentation rate of seaweed from 10 % (v/v) to 15 % (v/v) enhanced total omega-3 fatty acid content in BSFL. The highest ($p < 0.05$) polyunsaturated fatty acid content was detected in BSFL fed with fermented seaweed. The highest ($p < 0.05$) saturated fatty acid (SFA)

Table 3
Performance and the nutrient utilization of black soldier fly larvae fed with eight substrates in Experiment 1.

Substrate	PM	FO	KA	GS	SW	FO+ KA	FO+ GS	FO+ SW	p *value*
Final body weight (mg/larvae)	125.5 ± 12.8^{bc}	187.1 ± 16.3^{d}	80.1 ± 13.5^{a}	76.1 ± 9.8^{a}	102.4 ± 36.6^{ab}	152.4 ± 19.8^{c}	137.3 ± 11.4^{c}	154.5 ± 15.3^{c}	< 0.001
Body weight gain (mg/larvae)	19.9 ± 1.3^{c}	29.9 ± 0.1^{d}	12.3 ± 3.4^{ab}	11.4 ± 3.0^{a}	15.9 ± 1.4^{b}	24.2 ± 2.6^{d}	21.7 ± 2.9^{cd}	24.5 ± 0.31^{d}	< 0.001
FCR (g/g)	3.83 ± 0.5^{b}	2.49 ± 0.4^{a}	5.87 ± 0.5^{d}	6.07 ± 0.2^{d}	4.57 ± 0.3^{c}	3.04 ± 0.1^{ab}	3.3 ± 0.56^{b}	3.01 ± 0.5^{ab}	< 0.001
Waste reduction (%)	49.0 ± 0.8^{b}	50.9 ± 6.5^{bc}	27.9 ± 7.7^{a}	21.6 ± 4.9^{a}	30.0 ± 8.7^{a}	51.7 ± 3.9^{bc}	60.8 ± 6.1^{c}	46.9 ± 1.3^{b}	< 0.001
Bio conversion efficiency (%)	26.1 ± 4.6^{bc}	40.1 ± 1.5^{e}	17.1 ± 1.4^{a}	16.5 ± 2.6^{a}	21.9 ± 5.7^{ab}	32.9 ± 1.4^{d}	29.8 ± 1.4^{cd}	33.3 ± 1.9^{d}	< 0.001
Nitrogen conversion efficiency (%)	25.0 ± 5.5^{c}	13.0 ± 8.1^{abc}	50.3 ± 6.9^{d}	41.4 ± 5.6^{d}	20.7 ± 7.1^{bc}	8.6 ± 5.4^{ab}	14.2 ± 7.0^{abc}	2.8 ± 0.7^{a}	< 0.001
Survival rate (%)	72.48 ± 1.64^{cd}	89.45 ± 1.79^{f}	70.51 ± 0.54^{bc}	69.47 ± 1.55^{b}	67.45 ± 1.30^{a}	75.05 ± 0.15^{e}	73.0 ± 0.03^{d}	72.48 ± 0.03^{cd}	< 0.001

Values are expressed as mean ± standard deviation. PM: poultry manure (100 %); FO: fish offal; KA: *Kappaphycus alvarezii*; GS: *Gracilaria salicornia*; SW: *Sargassum wightii* (; FO+KA: Fish offal + *K. alvarezii*; FO+GS: Fish offal + *G. salicornia* (; FO+SW: Fish offal + *S. wightii*; FCR: feed conversion ratio. Values in the same row with different superscript letters are significantly different ($p < 0.05$) according to Duncan multiple range test. p value: p value for one way ANOVA.

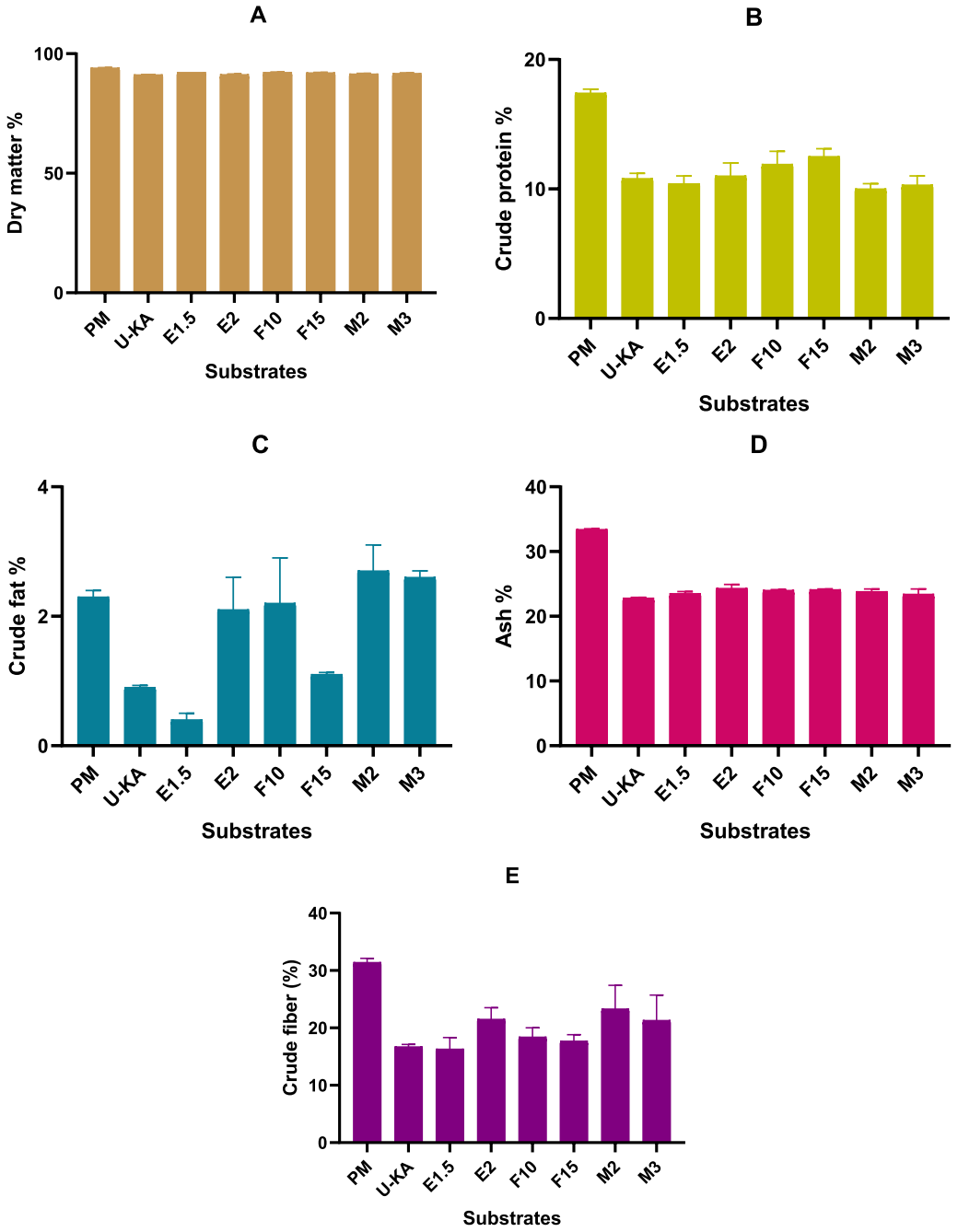

Fig. 5. Proximate composition (% dry matter) of eight substrates fed to black soldier fly larvae in Experiment 2. A: dry matter (%); B: crude protein; C: crude fat; D: ash; E: crude fiber; PM: poultry manure (100 %); U-KA: untreated *Kapphaphycus alvarezii*; E1.5: *K. alvarezii* treated with 1.5 % enzyme; E2: *K. alvarezii* treated with 2 % enzyme; F10: *K. alvarezii* fermented with 10 % yeast inoculum; F15: *K. alvarezii* fermented with 15 % yeast inoculum; M2: *K. alvarezii* microwaved for 2 min; M3: *K. alvarezii* microwaved for 3 min.

content was observed in BSFL fed with 100 % poultry manure, whereas BSFL fed with 2 % enzyme-treated seaweed and 10 % fermented seaweed had the lowest ($p < 0.05$) SFA content. Lauric acid (C12:0), pentadecanoic acid (C15:0), and stearic acid (C18:0) contents were higher ($p < 0.05$) in BSFL fed with 100 % poultry manure (Table 5). A positive correlation was observed between substrate total omega 3 contents and the corresponding levels in larvae (Fig. 8).

3.2.2. *Larval performance and nutrient utilization*

The performance and nutrient utilization of BSFL fed eight substrates are shown in Table 6. The inclusion of seaweed, whether

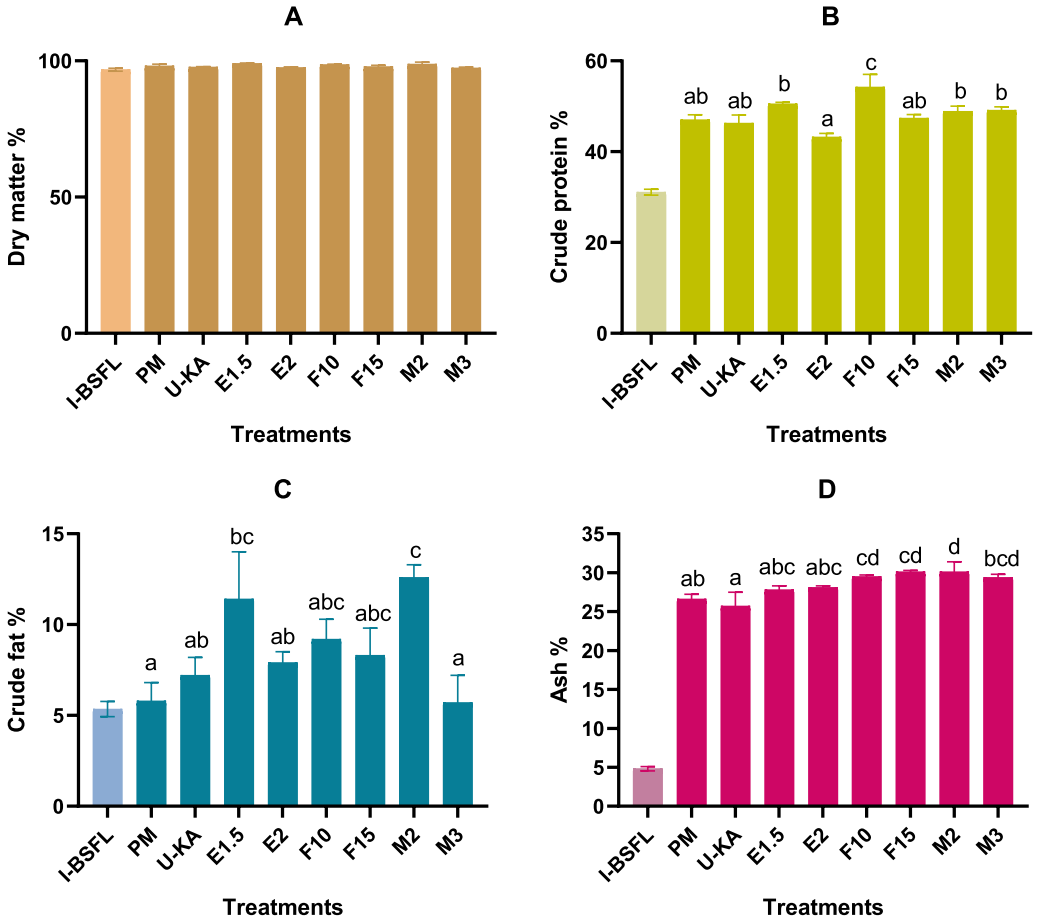

Fig. 6. Proximate composition (% dry matter) of black soldier fly (*Hermetia illucens*) larvae fed with eight substrates in Experiment 2. A: dry matter (%); B: crude protein; C: crude fat; D: ash; I-BSFL: initial black solder fly larvae; PM: poultry manure (100 %); U-KA: untreated *Kapphaphycus alvarezii*; E1.5: *K. alvarezii* treated with 1.5 % enzyme; E2: *K. alvarezii* treated with 2 % enzyme; F10: *K. alvarezii* fermented with 10 % yeast inoculum; F15: *K. alvarezii* fermented with 15 % yeast inoculum; M2: *K. alvarezii* microwaved for 2 min; M3: *K. alvarezii* microwaved for 3 min; SEM: standard error mean. Bars with different letters within each graph are significantly different ($p < 0.05$) according to Duncan multiple range test.

untreated or pre-treated, resulted in reductions in final body weight, body weight gain, waste reduction, bioconversion efficiency, and nitrogen conversion efficiency of the larvae, while increasing in FCR ($p < 0.05$). No significant differences were observed in these performance parameters between larvae fed untreated and pre-treated seaweed. However, some pre-treated seaweed supplemented substrates resulted in numerically higher values for these parameters compared to the untreated seaweed group. A similar trend was observed for FCR, except in larvae fed seaweed microwaved for 3 min, which exhibited a higher FCR ($p < 0.05$) than those fed untreated seaweed. Notably, survival rate was higher in larvae fed the substrate fermented with 15 % inoculum compared to other seaweed treatments. Additionally, there was a negative correlation ($p < 0.05$) between substrate total PUFA and waste reduction in larvae. No significant correlations were observed between larval fatty acid contents and other performance parameters (Figure S3).

4. Discussion

The nutrient composition of BSFL is known to vary with diet, and this study investigated how different substrate formulations, including seaweed, influence the nutrient content and performance of the larvae. The BSFL in the present study contained 35–54 % crude protein and 3–12 % crude fat on dry matter basis, and these fat contents were below the values reported in previous studies (Li et al., 2022). Incorporating seaweed into the substrates led to a further reduction in larval fat content, consistent with previous observations (Liland et al., 2017). Crude fat accumulation in BSFL was impacted by the reducing sugar content of the substrate (Truzzi et al., 2020). Seaweeds generally contain low levels of reducing sugars (Widyaningrum et al., 2016), which may contribute to the lower fat accumulation observed. Additionally, specific carbohydrate components found in seaweed, such as galactose, xylose, and arabinose (Xie et al., 2024), have been reported to negatively affect the fat content (Cohn et al., 2022). The rearing substrates also played a role in shaping the fatty acid composition of BSFL. In the experiment 1, irrespective of the substrate, SFA was found to be the main group of

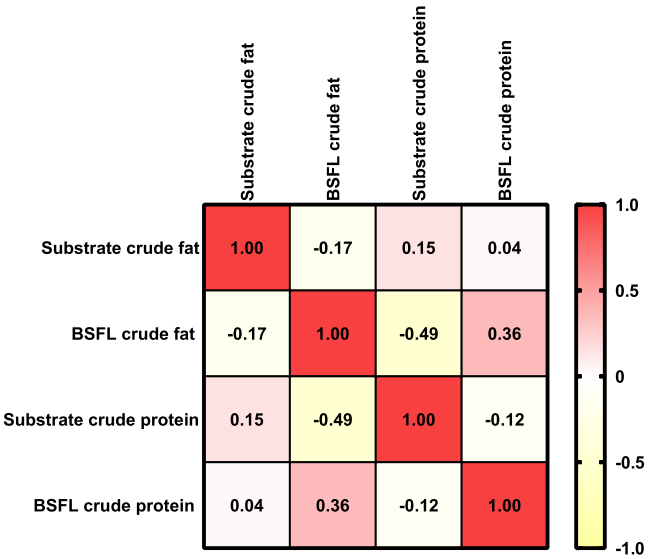

Fig. 7. Correlation matrix of protein and fat contents between black soldier fly larvae (BSFL; *Hermetia illucens*) and their substrates in Experiment 2. The Pearson correlation coefficients are shown numerically.

Table 4
Fatty acid composition (% of total fatty acids) of eight substrates fed to black soldier fly larvae in Experiment 2.

Fatty acid	*PM*	*U-KA*	*E1.5*	*E2*	*F10*	*F15*	*M2*	*M3*
C10:0	*0.8 ± 0.1*	*ND*	*2.6 ± 0.2*	*3.9 ± 0.3*	*2.8 ± 0.3*	*5.0 ± 0.1*	*16.5 ± 0.3*	*ND*
C12:0	*5.6 ± 0.3*	*ND*	*2.7 ± 0.4*	*5.7 ± 0.3*	*4.4 ± 0.2*	*1.6 ± 0.2*	*0.8 ± 0.2*	*0.5 ± 0.2*
C14:0	*2.4 ± 0.2*	*ND*	*2.4 ± 0.3*	*5.5 ± 0.2*	*4.3 ± 0.3*	*ND*	*ND*	*0.5 ± 0.2*
C15:0	*3.1 ± 0.2*	*ND*	*ND*	*14.8 ± 0.3*	*ND*	*ND*	*1.4 ± 0.3*	*1.4 ± 0.3*
C16:0	*1.5 ± 0.3*	*ND*	*7.5 ± 0.2*	*4.8 ± 0.3*	*12.4 ± 0.4*	*0.8 ± 0.1*	*1.4 ± 0.2*	*0.3 ± 0.2*
C16:1	*10.0 ± 0.3*	*ND*	*ND*	*ND*	*3.6 ± 0.5*	*ND*	*ND*	*ND*
C17:0	*ND*	*ND*	*ND*	*32.8 ± 0.4*	*24.4 ± 0.3*	*1.6 ± 0.2*	*2.6 ± 0.2*	*2.4 ± 0.2*
C18:0	*18.6 ± 0.2*	*ND*	*21.7 ± 0.2*	*6.1 ± 0.3*	*5.2 ± 0.4*	*2.1 ± 0.2*	*3.0 ± 0.2*	*21.0 ± 0.3*
C18:1 cis (n9)	*3.5 ± 0.1*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
C18:2 cis (n6)	*14.6 ± 0.2*	*27.1 ± 0.3*	*ND*	*ND*	*ND*	*26.6 ± 0.3*	*15.1 ± 0.3*	*ND*
C20:3n3	*ND*	*ND*	*5.2 ± 0.4*	*4.9 ± 0.4*	*12.4 ± 0.1*	*ND*	*ND*	*3.6 ± 0.2*
C20:5n3 (EPA)	*ND*	*47.1 ± 0.4*	*26.1 ± 0.9*	*8.1 ± 0.5*	*16.5 ± 0.3*	*46 ± 0.3*	*43.8 ± 0.3*	*40.4 ± 0.3*
C22:6n6 (DHA)	*ND*	*16.5 ± 0.2*	*22.6 ± 0.3*	*4.0 ± 0.3*	*10.3 ± 0.2*	*15.8 ± 0.4*	*15.5 ± 0.3*	*14.9 ± 0.1*
∑SFA	*51.9 ± 0.2*	*9.4 ± 0.2*	*46.0 ± 0.5*	*83.0 ± 0.6*	*57.1 ± 0.3*	*11.6 ± 0.2*	*25.6 ± 0.6*	*41.1 ± 0.4*
∑MUFA	*33.5 ± 0.4*	*ND*	*ND*	*ND*	*3.6 ± 0.3*	*ND*	*ND*	*ND*
∑PUFA	*14.6 ± 0.4*	*27.1 ± 0.5*	*ND*	*ND*	*ND*	*26.6 ± 0.3*	*15.1 ± 0.5*	*ND*
∑PUFA (n6)	*14.6 ± 0.4*	*27.1 ± 0.5*	*ND*	*ND*	*ND*	*26.6 ± 0.2*	*15.1 ± 0.3*	*ND*
∑PUFA (n3)	*ND*	*63.5 ± 0.5*	*54.0 ± 0.3*	*17.0 ± 0.3*	*39.2 ± 0.1*	*61.8 ± 0.5*	*59.2 ± 0.2*	*58.9 ± 0.5*

Values are expressed as mean ± standard deviation. PM: poultry manure (100 %); U-KA: untreated *Kapphaphycus alvarezii*; E1.5: *K. alvarezii* treated with 1.5 % enzyme; E2: *K. alvarezii* treated with 2 % enzyme; F10: *K. alvarezii* fermented with 10 % yeast inoculum; F15: *K. alvarezii* fermented with 15 % yeast inoculum; M2: *K. alvarezii* microwaved for 2 min; M3: *K. alvarezii* microwaved for 3 min; ND: not detected; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

fatty acids in BSFL. Particularly, regardless of substrate composition, all larvae exhibited high concentrations of lauric acid (C12:0), palmitic acid (C16:0) and oleic acid (C18:1 n9), even though these fatty acids were not abundant in the substrates. This suggests that BSFL may have synthesized these fatty acids *de novo* (Ewald et al., 2020). However, the lauric acid content was lower in larvae fed substrates containing fish offal and *K. alvarezii*. Omega-3 fatty acids were not detected in the initial larvae but were present at the end of the feeding period in larvae fed substrates containing fish offal or seaweed. Notably, the three substrataes that contained both seaweed and fish offal enriched larvae with EPA and DHA. *K. alvarezii* showed this enrichment even in the absence of fish offal. For *G. salicornia* and *S. wightii,* it is possible that the observed enrichment in EPA and DHA was influenced by the presence of fish offal in the substrate, These findings are in consistent with previous studies showing that omega-3 enrichment in BSFL is possible through supplementation of substrates with fish offal (St-Hilaire et al., 2007a, 2007b) and various seaweeds, including *Laminaria digitata*, *Fucus serratus*, *Palmaria palmata*, *Ulva lactuca* (Swinscoe et al., 2020) and *Ascophyllum nodosum* (Liland et al., 2017). Present results further showed that larvae reared on substrates containing 100 % poultry manure or 12 % *S. wightii* or *G. salicornia* did not exhibit any omega-3 enrichment, as these substrates lacked omega-3 fatty acids. This indicates that dietary inclusion of omega-3 sources is necessary for their deposition in BSFL. Correlation analyses further supported a positive relationship between omega-3 fatty acid levels

Table 5
Fatty acid composition (% of total fatty acids) of black soldier fly larvae fed eight substrates in Experiment 2.

Fatty acid	*I-BSFL*	*PM*	*U-KA*	*E1.5*	*E2*	*F10*	*F15*	*M2*	*M3*	p value
C10:0	*0.7 ± 0.0*	*0.7 ± 0.0^{d}*	*0.3 ± 0.0^{c}*	*0.2 ± 0.0^{b}*	*0.1 ± 0.0^{a}*	*0.7 ± 0.0^{d}*	*0.9 ± 0.1^{e}*	*3.0 ± 0.0^{g}*	*1.9 ± 0.0^{f}*	*< 0.001*
C12:0	*28.8 ± 0.7*	*11.3 ± 0.0^{h}*	*4.2 ± 0.0^{f}*	*2.0 ± 0.0^{a}*	*2.4 ± 0.0^{c}*	*2.0 ± 0.0^{b}*	*2.7 ± 0.0^{d}*	*3.8 ± 0.0^{e}*	*4.8 ± 0.0^{g}*	*< 0.001*
C13:0	*ND*	*3.0 ± 0.0^{f}*	*1.0 ± 0.0^{c}*	*1.3 ± 0.1^{d}*	*0.2 ± 0.0^{a}*	*0.3 ± 0.0^{b}*	*0.2 ± 0.0^{a}*	*2.3 ± 0.0^{e}*	*0.2 ± 0.0^{a}*	*< 0.001*
C14:0	*9.1 ± 0.1*	*3.8 ± 0.0^{f}*	*3.9 ± 0.0^{g}*	*3.7 ± 0.0^{e}*	*2.7 ± 0.0^{c}*	*3.6 ± 0.0^{d}*	*2.0 ± 0.0^{b}*	*< 0.01 ± 0.00^{a}*	*2.7 ± 0.0^{c}*	*< 0.001*
C14:1	*ND*	*2.7 ± 0.0*	*2.5 ± 0.0^{c}*	*4.0±0.0^{h}*	*3.5 ± 0.0^{f}*	*3.6 ± 0.0^{g}*	*2.2 ± 0.0^{a}*	*3.0 ± 0.0^{e}*	*2.3 ± 0.0^{b}*	*< 0.001*
C15:0	*0.10 ± 0.02*	*5.8 ± 0.0^{e}*	*3.2 ± 0.1^{c}*	*4.0 ± 0.0^{d}*	*2.7 ± 0.2^{b}*	*4.0 ± 0.4^{d}*	*2.6 ± 0.1^{b}*	*< 0.01 ± 0.00^{a}*	*2.7 ± 0.2^{b}*	*< 0.001*
C16:0	*23.2 ± 0.1*	*12.5 ± 0.0^{c}*	*12.0 ± 0.1^{b}*	*15.6 ± 0.1^{e}*	*13.6 ± 0.1^{d}*	*15.5 ± 0.1^{e}*	*11.8 ± 0.0^{b}*	*11.1 ± 0.6^{a}*	*15.3 ± 0.3^{e}*	*< 0.001*
C16:1	*0.4 ± 0.1*	*0.4 ± 0.0^{c}*	*0.5 ± 0.0^{d}*	*3.0 ± 0.0^{g}*	*2.8 ± 0.1^{f}*	*0.4 ± 0.0^{bc}*	*1.5 ± 0.1^{e}*	*< 0.01 ± 0.00^{a}*	*0.3 ± 0.0^{b}*	*< 0.001*
C17:0	*2.0 ± 0.1*	*2.9 ± 0.1^{b}*	*10.5 ± 0.1^{e}*	*15.3 ± 0.0^{g}*	*10.8 ± 0.0 ^{f}*	*2.1 ± 0.0^{a}*	*8.9 ± 0.0^{d}*	*7.6 ± 0.0^{c}*	*10.7 ± 0.1^{f}*	*< 0.001*
C18:0	*5.3 ± 0.1*	*16.7 ± 0.1^{f}*	*1.8 ± 0.0^{b}*	*2.6 ± 0.2^{c}*	*5.6 ± 0.1^{d}*	*1.7 ± 0.1^{b}*	*1.8 ± 0.3^{b}*	*< 0.01 ± 0.00^{a}*	*7.6 ± 0.1^{e}*	*< 0.001*
C18:1 cis(n9)	*24.4 ± 0.1*	*18.7 ± 0.1^{d}*	*13.0 ± 0.2^{c}*	*17.8 ± 1.3^{d}*	*28.2 ± 0.3^{f}*	*8.8 ± 0.1^{b}*	*7.2 ± 0.1^{a}*	*22.3 ± 0.2^{e}*	*18.6 ± 0.1^{d}*	*< 0.001*
C18:2 cis (n6)	*1.3 ± 0.0*	*3.6 ± 0.1^{c}*	*0.2 ± 0.0^{a}*	*0.8 ± 0.1^{b}*	*3.8^{c}±0.0*	*19.4 ± 0.1^{f}*	*14.2 ± 0.3^{e}*	*< 0.01 ± 0.00^{a}*	*4.4 ± 0.2^{d}*	*< 0.001*
C20:0	*ND*	*4.7 ± 0.1^{c}*	*0.7 ± 0.1^{a}*	*11.3 ± 0.6^{f}*	*0.5 ± 0.1^{a}*	*6.0 ± 0.1^{d}*	*4.0 ± 0.2^{b}*	*10.8 ± 0.1^{e}*	*15.5 ± 0.2^{g}*	*< 0.001*
C18:3n6	*1.4 ± 0.1*	*1.5 ± 0.1^{c}*	*7.2 ± 0.1^{e}*	*2.2 ± 0.0^{d}*	*15.3 ± 0.0^{h}*	*13.2 ± 0.1^{g}*	*9.3 ± 0.1^{f}*	*< 0.01 ± 0.00^{a}*	*0.8 ± 0.1^{b}*	*< 0.001*
C20:1n9	*ND*	*0.6 ± 0.0^{c}*	*4.1 ± 0.1^{e}*	*0.3 ± 0.0^{b}*	*0.3 ± 0.0^{b}*	*0.8 ± 0.0^{d}*	*< 0.01 ± 0.00^{a}*	*< 0.01 ± 0.00^{a}*	*< 0.01 ± 0.00^{a}*	*< 0.001*
C18:3n3	*ND*	*< 0.01 ± 0.0^{a}*	*0.9 ± 0.1^{d}*	*0.7 ± 0.1^{bc}*	*0.7 ± 0.0^{b}*	*0.7 ± 0.0^{b}*	*6.0 ± 0.0^{e}*	*3.4 ± 0.0^{f}*	*0.8 ± 0.1^{c}*	*< 0.001*
C22:0	*0.6 ± 0.1*	*1.4 ± 0.1^{d}*	*0.4 ± 0.0^{a}*	*0.7 ± 0.0^{c}*	*0.3 ± 0.1^{a}*	*3.0 ± 0.1^{g}*	*2.0 ± 0.1^{e}*	*2.7 ± 0.1^{f}*	*0.6 ± 0.1^{b}*	*< 0.001*
C20:3n3	*ND*	*< 0.01 ± 0.00^{a}*	*2.0 ± 0.1^{d}*	*3.5 ± 0.1^{f}*	*1.8 ± 0.1^{d}*	*0.6 ± 0.1^{b}*	*1.0 ± 0.1^{c}*	*1.9 ± 0.2^{d}*	*2.9 ± 0.1^{e}*	*< 0.001*
C23:0	*ND*	*2.0 ± 0.1^{d}*	*2.5 ± 0.1^{e}*	*< 0.01 ± 0.00^{a}*	*0.4 ± 0.0^{b}*	*< 0.01 ± 0.00^{a}*	*< 0.01 ± 0.00^{a}*	*18.7 ± 0.0^{f}*	*0.6 ± 0.0^{c}*	*< 0.001*
C20:5n3 (EPA)	*ND*	*< 0.01 ± 0.00^{a}*	*1.4 ± 0.1^{c}*	*2.3 ± 0.1^{f}*	*0.8 ± 0.1^{b}*	*1.5 ± 0.0^{d}*	*1.6 ± 0.0^{e}*	*3.8 ± 0.0^{g}*	*1.4 ± 0.0^{cd}*	*< 0.001*
C24:0	*ND*	*< 0.01 ± 0.00^{a}*	*11.8 ± 0.1^{f}*	*0.2 ± 0.0^{b}*	*0.8 ± 0.0^{c}*	*1.5 ± 0.1^{d}*	*11.5 ± 0.1^{e}*	*< 0.01 ± 0.00^{a}*	*< 0.01 ± 0.00^{a}*	*< 0.001*
C22:6n3 (DHA)	*ND*	*< 0.01 ± 0.00^{a}*	*4.2 ± 0.5^{g}*	*2.2 ± 0.1^{e}*	*0.9 ± 0.1^{b}*	*1.3 ± 0.1^{c}*	*3.8 ± 0.1^{f}*	*1.7 ± 0.1^{d}*	*1.0 ±.1^{b}*	*< 0.001*
∑ SFA	*72.3 ± 1.3*	*65.4 ± 0.7^{g}*	*52.7 ± 0.8^{c}*	*59.3 ± 0.0^{d}*	*40.8 ± 0.1^{a}*	*40.8 ± 0.4^{a}*	*48.9 ± 0.9^{b}*	*63.8 ± 1.1^{f}*	*62.6 ± 0.4^{e}*	*< 0.001*
∑ MUFA	*25.1 ± 0.2*	*29.5 ± 0.5^{d}*	*27.5 ± 0.6^{cd}*	*29.0 ± 1.9^{d}*	*35.9 ± 3.0^{e}*	*22.6 ± 0.8^{b}*	*15.1 ± 0.2^{a}*	*25.4 ± 0.6^{c}*	*26.3 ± 0.3^{c}*	*< 0.001*
∑ PUFA	*2.6 ± 0.0*	*5.1 ± 0.1^{a}*	*19.7 ± 0.2^{c}*	*11.7 ± 0.1^{b}*	*23.3 ± 1.6^{d}*	*36.7 ± 2.0^{e}*	*36.0 ± 0.5^{e}*	*10.9 ± 0.3^{b}*	*11.2 ± 0.6^{b}*	*< 0.001*
∑PUFA (n3)	*ND*	*< 0.01 ± 0.00^{a}*	*8.5 ± 0.1^{d}*	*8.6 ± 0.1^{d}*	*4.2 ± 0.2^{b}*	*4.0 ± 0.1^{b}*	*12.4 ± 0.0^{f}*	*10.9 ± 0.2^{e}*	*6.0 ± 0.1^{c}*	*< 0.001*
∑PUFA(n6)	*2.6 ± 0.1*	*5.1 ± 0.4^{c}*	*11.2 ± 0.5^{d}*	*3.0 ± 0.5^{b}*	*19.1 ± 0.4^{e}*	*32.7 ± 0.3^{g}*	*23.6 ± 0.2^{f}*	*< 0.01 ± 0.00^{a}*	*5.2 ± 0.4^{c}*	*< 0.001*

I-BSFL: initial black solider fly larvae; PM: poultry manure (100 %); U-KA: untreated *Kapphaphycus alvarezii*; E1.5: *K. alvarezii* treated with 1.5 % enzyme; E2 *K. alvarezii* treated with 2 % enzyme; F10: *K. alvarezii* fermented with 10 % yeast inoculum; F15: *K. alvarezii* fermented with 15 % yeast inoculum; M2 *K. alvarezii* microwaved for 2 min; M3: *K. alvarezii* microwaved for 3 min; ND: not detected; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Values in the same row with different superscript letters are significantly different ($p < 0.05$) according to Duncan multiple range test. p value: p value for one way ANOVA.

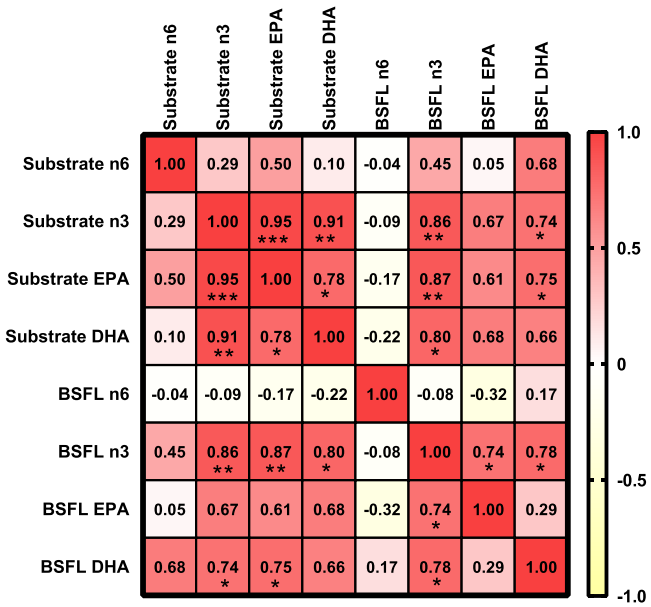

Fig. 8. Correlation matrix between the fatty acid contents (% of total fatty acids) of black soldier fly larvae (BSFL; *Hermetia illucens*) and the corresponding fatty acid contents (% of total fatty acids) in their substrates in Experiment 2. The Pearson correlation coefficients are shown numerically, with asterisks indicating significance levels (*p < 0.05; **p < 0.01; ***p < 0.001). n6: total omega 6 fatty acids; n3: total omega 3 fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

in the substrate and larvae, consistent with previous observations (Ewald et al., 2020). Seaweed lipid content and fatty acid composition can vary considerably based on genotype and external conditions like light intensity, temperature, and nutrient availability (Breeman, 1990; Jayasinghe et al., 2018). The observed differences in fatty acid profiles among substrates may be attributed to species-specific traits of seaweeds, which in turn may affect the larvae fed on them. The substrate type is further a key determinant of performance and nutrient utilization efficiency of BSFL (Opoku et al., 2023). Nutrient-dense substrates have been shown to enhance growth rates, survival rate and bioconversion efficiency in BSFL compared to substrates with lower nutritional value (Albalawneh et al., 2024; Beniers et al., 2019). In particular, BSFL fed protein-rich substrates typically exhibit superior growth and feed conversion efficiency (Eggink et al., 2023). Insufficient energy in the substrate can force the larvae to catabolize dietary protein to meet their energy demands, ultimately compromising growth performance (Eggink et al., 2022). In this study, the higher performance of larvae reared on fish offal-based substrates can likely be due to the high protein content of the substrate. Despite the omega-3 enrichment, the inclusion of seaweed in the substrates negatively affected larval performance. Liland et al. (2017) also reported that seaweed inclusion levels above 50 % reduced the protein and lipid content, survival rate and the growth of the larvae. One possible explanation for this is the lower protein content in seaweed-supplemented substrates. Additionally, seaweed contains only a small proportion of lipids (1–5 %) (Morais et al., 2020), and dietary fats are crucial for the build up of lipids in BSFL, which are vital for energy reserves and development (Albalawneh et al., 2024). Furthermore, seaweed is relatively difficult to digest due to its high fiber and phlorotannin content (Gregersen et al., 2021). Brown algae cell walls contain fucose-based sulfated polysaccharides (fucoidans), which form complex and dynamic structures (Deniaud-Bouët et al., 2017; Torode et al., 2015). High contents of structural fiber and fucoidans may decrease nutrient availability and digestibility (Gregersen et al., 2021), potentially impairing BSFL growth and nutrient absorption (Liland et al., 2017). Certain carbohydrates such as galactose, xylose, and arabinose present in seaweed (Xie et al., 2024), are also associated with reduced larval growth (Cohn et al., 2022). Soluble carbohydrates like maltose and sucrose have been shown to enhance BSFL growth performance compared to cellulose (Carpentier et al., 2024). The low soluble carbohydrate content in seaweed may therefore further contribute to the observed reductions in larval performance. Moreover, waste reduction of BSFL is known to depend on substrate composition (Opoku et al., 2023), with crude fiber and lignin contents decrease the waste reduction ratio (Adi Rohmanna and Maulidya, 2022). The lower waste reduction observed in larvae fed seaweed substrates could be attributed to the increased fiber content resulting from seaweed inclusion.

Processing of seaweed can improve their nutritional quality and bioavailability by disrupting cell wall structures and enhancing the release of bioactive compounds (Aarthy et al., 2018; Campbell et al., 2020; Fernandes et al., 2022). For instance, processing techniques like microwave drying (Romagnoli et al., 2017), fermentation (Hardjani et al., 2017), and enzymatic treatments (Matshogo et al., 2021), have been shown to enhance seaweed digestibility and nutrient availability. Accordingly, the second experiment in this study was conducted to investigate whether supplementing substrates with pre-treated seaweed can enhance both nutritional value and growth performance of BSFL.

The inclusion of seaweed in the substrates, irrespective of the pre-treatment method, resulted in a slight reduction in protein content. In contrast, the incorporation of pre-treated seaweed led to an increase in fat content. Notably, omega-3 fatty acids were

Table 6
Performance and nutrient utilization of black solder fly larvae fed with eight substrates in Experiment 2.

Substrate	PM	U-KA	E1.5	E2	F10	F15	M2	M3	*p* value
Final body weight (mg/larvae)	63.1 ± 6.8^{c}	20.6 ± 2.9^{ab}	21.7 ± 3.9^{ab}	26.3 ± 9.0^{b}	22.0 ± 2.3^{ab}	20.4 ± 3.8^{ab}	23.3 ± 1.8^{ab}	15.3 ± 2.4^{a}	< 0.001
Body weight gain (mg/larvae)	58.8 ± 7.1^{c}	16.4 ± 3.8^{ab}	17.4 ± 3.7^{ab}	22.1 ± 8.6^{b}	17.9 ± 1.9^{ab}	16.1 ± 3.6^{ab}	19.0 ± 2.4^{ab}	11.0 ± 3^{b}	< 0.001
Feed conversion ratio (g/g)	14.4 ± 1.6^{a}	51.8 ± 12.8^{b}	48.2 ± 11.3^{b}	42.5 ± 21.0^{b}	46.0 ± 5.0^{b}	53.1 ± 12.7^{b}	43.0 ± 5.4^{b}	78.1 ± 18.8^{c}	0.002
Waste reduction (%)	54.6 ± 4.4^{b}	28.3 ± 8.2^{a}	32.9 ± 5.9^{a}	34.4 ± 1.6^{a}	31.2 ± 1.1^{a}	26.7 ± 4.4^{a}	29.5 ± 5.5^{a}	30.0 ± 3.3^{a}	< 0.001
Nitrogen conversion efficiency (%)	25.1 ± 2.3^{c}	11.7 ± 1.9^{ab}	14.4 ± 2.7^{ab}	14.3 ± 5.8^{ab}	13.8 ± 0.3^{ab}	10.2 ± 1.8^{a}	15.8 ± 2.2^{b}	9.1 ± 2.3^{a}	< 0.001
Bioconversion efficiency (%)	7.0 ± 0.8^{c}	2.0 ± 0.5^{ab}	2.1 ± 0.5^{ab}	2.7 ± 1.1^{ab}	2.2 ± 0.2^{b}	2.0 ± 0.4^{ab}	2.3 ± 0.3^{ab}	1.3 ± 0.4^{a}	< 0.001
Survival rate (%)	75.2 ± 5.6^{abc}	64.3 ± 9.7^{ab}	78.3 ± 15.3^{abc}	74.2 ± 5.7^{abc}	80.4 ± 3.6^{abc}	94.1 ± 1.3^{c}	62.3 ± 20.3^{a}	84.5 ± 11.4^{bc}	0.047

PM: poultry manure (100 %); U-KA untreated *Kapphaphycus alvarezii*; E1.5: *K. alvarezii* treated with 1.5 % enzyme; E2: *K. alvarezii* treated with 2 % enzyme; F10: *K. alvarezii* fermented with 10 % yeast inoculum; F15: *K. alvarezii* fermented with 15 % yeast inoculum; M2: *K. alvarezii* microwaved for 2 min; M3: *K. alvarezii* microwaved for 3 min. Values in the same row with different superscript letters are significantly different ($p < 0.05$) according to Duncan multiple range test

absent in the control substrate without seaweed but were detectable in all seaweed-supplemented substrates, regardless of treatment. Seaweed supplementation, regardless of pre-treatment, resulted in reductions in final body weight, weight gain, waste reduction, nitrogen conversion efficiency, and bioconversion efficiency, while FCR was increased. The performance values observed in this study were lower than those typically reported for BSFL reared on other waste substrates (Adi Rohmanna and Maulidya, 2022). This may be attributed to several factors, including the lower initial larval weight at the start of the experiment compared to previous studies, as well as the high inclusion levels of seaweed, which may have introduced elevated amounts of anti-nutritional compounds and salt content inherent to seaweed. However, when compared with Liland et al. (2017), the final larval weights observed in our study were comparable for larvae reared on diets with 60 % seaweed inclusion. Despite the overall reductions, larvae reared on pre-treated seaweed substrates showed numerically better performance metrics than those given untreated seaweed, indicating that pre-treatment may have the potential to alleviate some of the negative impacts of seaweed supplementation. However, when considering survival rate, our results showed a comparatively higher survival in the treatment with 15 % fermented seaweed, suggesting that fermentation may improve larval resilience and viability.

A clear dose-dependent trend was observed, with increasing levels of enzyme treatment of seaweed from 1.5 % to 2 % corresponding to decreased concentrations of EPA, DHA, and total omega-3 fatty acids in larvae. The enzyme mixture used (Allzyme®) contained protease, phytase, β-glucanase, xylanase, cellulase, and amylase. Phytase can enhance nutrient digestibility and reduces anti-nutritional factors, and protease promotes microbial degradation of organic matter (Furuya et al., 2023). Hence, increased enzyme activity may accelerate microbial activity in the substrate, resulting in microbes consuming readily available nutrients and leaving behind more complex, less accessible compounds for the larvae (Siva Raman et al., 2022). This microbial competition could explain the observed decline in accumulation of omega-3 fatty acids in BSFL reared on 2 % enzyme-treated substrates.

Fermentation, particularly with a 15 % inoculum, enhanced EPA and total omega-3 fatty acid levels in the larvae compared to those fed untreated seaweed. This effect may be attributed to the yeast used in fermentation, which is known to synthesize long-chain fatty acids involved in membrane formation, energy storage, and protein modification (Hardjani et al., 2017). Some of these yeast-derived compounds can be utilized by BSFL, potentially contributing to increased omega-3 content.

Microwave treatment for 2 min increased EPA and total omega-3 fatty acid levels in the larvae. However, extending the treatment duration to 3 min adversely affected the accumulation of EPA, DHA, and total omega-3s in BSFL. Microwave processing is known to elevate internal temperature and pressure within biomass, disrupting cellular structures and thereby enhancing nutrient release and bioavailability (Bandici et al., 2022). Although microwave treatment can enhance nutrient accessibility for BSFL, it may also elevate the risk of microbial growth by increasing the availability of easily digestible compounds. Microorganisms present in the substrate may outcompete the larvae for these nutrients, potentially reducing the accumulation of omega-3 fatty acids in the larvae. This microbial competition may partly explain the decline in omega-3 levels observed with prolonged microwave exposure. Therefore, while microwave treatment holds promise for improving omega-3 deposition in BSFL, careful optimization of exposure time is crucial to balance nutrient availability and microbial activity.

Previous studies have documented a positive correlation between larval SFA content and performance, while performance tends to negatively correlate with PUFA and MUFA contents (Ewald et al., 2020). These findings suggest that attempts to alter the fatty acid profile of BSFL may be constrained by a trade-off with growth performance. However, in the present study, no such trade-offs were observed between PUFA, MUFA, and performance, indicating that fatty acid profile modification was not limited by growth performance when pre-treated seaweed was used.

5. Conclusion

The present findings confirm the nutritional plasticity of BSFL, particularly in terms of lipid content and fatty acid composition. Feeding seaweed (*K. alvarezii*, *G. salicornia*, and *S. wightii*) together with fish offal enriched the larvae with EPA and DHA, while *K. alvarezii* showed this enrichment even in the absence of fish offal. Notably, pre-treatment of *K. alvarezii*, particularly through fermentation and microwave drying, further increased its capacity to elevate omega-3 content in the larvae compared to its untreated form. Although seaweed supplementation generally reduced larval growth performance, seaweed pre-treated with enzymes, fermentation, or microwave drying demonstrated numerical improvements in performance indicators than untreated seaweed. Overall, although seaweed supplementation may enrich BSFL with beneficial fatty acids, particularly omega-3, it compromises growth and conversion efficiencies. Future research should explore synergistic strategies, such as incorporating specific microbiomes capable of breaking down seaweed's complex polysaccharides, to enhance the bioavailability of nutrients and improve overall BSFL performance.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT in order to improve readability and language of some sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2025.116542.

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