

Hermetia illucens Larvae Meal as Partial Fishmeal Replacement in Jade Perch Diet Cultured in Biofloc: Growth Performance, Feed Efficiency and Consumer Acceptance

(Tepung Larva *Hermetia illucens* sebagai Pengganti Separa Tepung Ikan dalam Diet Jade Perch yang Dikultur dalam Bioflok: Prestasi Pertumbuhan, Kecekapan Makanan dan Penerimaan Pengguna)

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ABSTRACT

A 12-week feeding trial on jade perch (*Scortum barcoo*) was conducted to evaluate the outcomes of partial substitution of fishmeal with black soldier fly larvae meal (BSFLM) in a biofloc system that requires minimal water exchange. The jade perch juveniles were subjected to three dietary treatments namely the 100% fishmeal (FM100), 50% BSFLM (BSF50), and a commercial diet (COM) with 20 individuals per treatment in triplicate tanks. Growth, feed efficiency, and body indices were measured throughout the feeding trial. There were no significant differences observed between the treatments in growth indicators such as the body weight gain (84.55 - 89.10 g), feed conversion ratio (1.77 - 1.87), specific growth rate (1.71 - 1.81 %/day), protein efficiency ratio (1.69 - 1.77), survival rate (90.00 - 98.48 %) and fillet yield (34.94 - 37.08 %). The condition factor is similar in all treatment groups ranging from 1.97 - 2.05. The crude protein and moisture content making up fish body composition are not statistically different among treatments, but higher crude lipids are obtained in the BSF50 (32.33 ± 5.27 g/kg) and COM (31.67 ± 2.76 g/kg). Higher hepatosomatic index (HSI) and visceral fat somatic index (VFSI) values were observed in BSF50 group at 3.13 ± 0.25 and 10.89 ± 1.68, respectively. Furthermore, sensory evaluation comparing the consumers' evaluation and acceptance of the fish cultured with the different dietary treatment and culture system shows that the group fed BSF50 and cultured in biofloc system received similar overall appreciation with the FM100 and COM group. In conclusion, BSFLM inclusion in the jade perch diet, combined with the biofloc system, is feasible to be explored further.

Keywords: Alternative feed; aquatic animals; biofloc technology; insect protein; sustainable aquaculture

ABSTRAK

Suatu kajian pemakanan selama 12 minggu ke atas ikan *jade perch* (*Scortum barcoo*) telah dijalankan untuk menilai hasil daripada penggantian separa tepung ikan dengan tepung larva lalat askar hitam (BSFLM) dalam sistem bioflok yang memerlukan pertukaran air yang minimum. Juvenil ikan jade perch telah diberikan tiga rawatan pemakanan iaitu 100% tepung ikan (FM100), 50% BSFLM (BSF50) dan diet komersial (COM) dengan 20 ekor individu untuk setiap rawatan dalam tiga kali pengulangan tangki. Pertumbuhan, kecekapan pemakanan, dan indeks badan telah diukur sepanjang ujian pemakanan. Tiada perbezaan yang signifikan diperhatikan antara rawatan tersebut dalam indikator pertumbuhan seperti pertambahan berat badan (84.55 - 89.10), nisbah penukaran makanan (1.77 - 1.87), kadar pertumbuhan khusus (1.71 - 1.81 %/day), nisbah kecekapan protein (1.69 - 1.77), kadar kemandirian (90.00 - 98.48%) dan hasil filet (34.94 - 37.08%). Faktor keadaan adalah serupa dalam semua kumpulan rawatan dengan julat 1.97 - 2.05. Kandungan protein kasar dan lembapan yang membentuk komposisi badan ikan tidak berbeza secara statistik antara rawatan, tetapi lemak kasar yang lebih tinggi diperoleh dalam BSF50 (32.33 ± 5.27 g/kg) dan COM (31.67 ± 2.76 g/kg). Nilai indeks

hepatosomatik (HSI) dan indeks somatik lemak visceral (VFSI) yang lebih tinggi telah diperhatikan dalam kumpulan BSF50 masing – masing pada 3.13 ± 0.25 dan 10.89 ± 1.68 . Selain itu, penilaian deria yang membandingkan penilaian dan penerimaan pengguna terhadap ikan yang dibiakkan dengan rawatan pemakanan dan sistem penternakan yang berbeza menunjukkan bahawa kumpulan yang diberi BSF50 dan dibiakkan dalam sistem bioflok menerima penghargaan keseluruhan yang serupa dengan kumpulan FM100 dan COM. Kesimpulannya, penggunaan BSFLM dalam diet jade perch, yang digabungkan dengan sistem bioflok, adalah wajar untuk dikaji lebih lanjut.

Kata kunci: Akuakultur mampan; haiwan akuatik; makanan alternatif; protein serangga; teknologi bioflok

INTRODUCTION

Jade perch (*Scortum barcoo*) that originates from Australia is becoming a high-demand aquaculture species that is widely cultured in China, Malaysia, and Singapore due to its exquisite taste, nutritional value, and the ability to thrive in the recirculating system and intensive ponds (Xie et al. 2021). Although the aquaculture sector contributes significantly to protein consumption worldwide, it comes with a price. Seventy-five percent of crustacean and finfish aquaculture production depends on feed (Boyd, McNevin & Davis 2022). With the sector intensifying, the fishmeal price also increases to meet the demand due to the depleted global fisheries stock. In addition, the expansion of this sector also calls for effective aquacultural waste management, as fish rearing will produce nutrient effluent, which, if not properly managed, will have environmental impacts. Scientists are searching for fishmeal alternatives and cost-effective techniques such as biofloc technology (BFT) for aquacultural waste management to foster more sustainable aquaculture.

Although it has been almost 50 years ago that insects have been appraised as the potential animal feedstuff, they have only been gaining attention recently as fish feed ingredients (Barroso et al. 2014). The black soldier fly (BSF) is a potential candidate as a sustainable aquafeed ingredient because of its richness in protein, short life cycle and can be reared on organic waste, thus promoting a circular economy and low production cost. The fly cannot transmit parasite or diseases although reared on waste materials (Siva Raman et al. 2022; van Huis et al. 2020). Moreover, BSF is one of the approved insect species allowed for animal protein production use in aquafeed by the European Union (EU) among the other species (Xu et al. 2020). BSF's protein and fat composition can range from 37 - 63% DM and 7 - 39% DM, respectively (Barragan-Fonseca, Dicke & van Loon 2017). Barroso et al. (2014) have highlighted that being a dipteran, the amino acid profile of black soldier fly larvae is significantly akin to that of fishmeal.

Numerous studies have been conducted to test the suitability of using BSF as fish feed ingredients. Vongvichith et al. (2020) reported that complete replacement of FM with BSF in climbing perch diet was

possible with reduced protein content (up to 25% crude protein) in feed without affecting fish final weight and crude protein with improved protein retention, and protein efficiency ratio. Moreover, in addition to the similar growth performance with the control, 50% fishmeal replacement with defatted BSF in the pikeperch diet was able to improve environmental impact with a reduction of fish-in-fish out (FIFO) ratio by 40.1% (Stejskal et al. 2023). Meanwhile, regarding sensory analysis of fish fed dietary BSF, Belgit et al. (2019) have also demonstrated that up to 100% fishmeal replacement with BSFL meal caused slight differences in the Atlantic salmon diets with no adverse effects on its growth performance.

Biofloc technology is a culture system in which waste from fish feces, urine, and excess feed during feeding are recycled into beneficial microbial protein biomass while clearing the water from inorganic nitrogen that is toxic to fish (Abakari, Luo & Kombat 2021; Yu et al. 2023). Biofloc systems can either be *in-situ* (cultured directly with target organisms) or *ex-situ* (incorporated as feed ingredients) (Deng et al. 2022; Menaga et al. 2019). *In-situ* biofloc is established by creating a high carbon-to-nitrogen ratio in the water column starting from 10:1 (Dong et al. 2021; Panigrahi et al. 2019) by an external supply of organic carbon sources in water or increasing carbon content in feed (Li et al. 2023) with a constant flow of aeration. This system benefits from minimal water change, reduced artificial feed input, and a natural establishment of microbial biomass (Mugwanya et al. 2021). Moreover, Nile tilapia cultured in *in-situ* live biofloc have shown to have growth-promoting effects due to the different gut microbiota communities that are beneficial compared to *ex-situ* biofloc and non-biofloc cultures (Deng et al. 2022).

Previous studies conducted on jade perch's growth performance include the effect of low salinity environment (Luo, Zhang & Tan 2012), feeding frequencies (Al-Khafaji et al. 2017) the substitution of fish oil with *Schizochytrium* sp. meal and vegetable oils (Van Hoestenberghes et al. 2016, 2013) and dietary lipid level (Song, Zhu & Wang 2009). However, limited studies are available regarding sensory evaluation of jade perch fed dietary BSF. The present study is the first to evaluate the effect of partial fishmeal replacement with dietary black soldier fly larvae meal

(BSFLM) on the growth performance, fillet composition, organosomatic indices, and consumers' acceptance of the sensory traits of jade perch cultured in biofloc systems.

MATERIALS AND METHODS

PREPARATION OF BLACK SOLDIER FLY LARVAE MEAL FOR THE FEED INGREDIENT

The black soldier fly larvae (BSFL) cultivation method follows Taufek, Lim and Bakar (2021) using soybean curd residue as the substrate for a period of 20 days. BSF eggs and the substrate in this study were purchased from a local supplier and cultured in Mini Farm, at Ladang Mini, Institute of Biological Sciences, Faculty of Science, Universiti Malaya. The eggs were weighed and transferred into bins containing the substrate by putting them on a small piece of paper placed on top of the substrate. This act prevented the eggs from contacting substrate moisture which can spoil them - the paper acted as a medium for neonates to get to the substrate only when they successfully hatched. The substrate was kept at ten-fold the weight of the eggs. Approximately 4 kg of dried BSFLM was produced in this study. The BSFL were fed soybean curd residue before being harvested after 20 days during the beginning of their pre-pupae. After the harvesting process, the pre-pupae were weighed and frozen at -20 °C before proximate composition analysis to determine their protein and fat content before being included in feed formulation. The frozen pre-pupae were thawed, dried at 60 °C overnight, ground into powder, and stored at 4 °C prior to the proximate analysis.

EXPERIMENTAL FEED PREPARATION

In this experiment, the jade perch fingerlings were subjected to three dietary treatments during the feeding trial. One commercial diet (Dindings Soya & Multifeeds Sdn. Bhd., Kuala Lumpur, Malaysia, CP: 32%; CL: 5%) and two isonitrogenous and isolipidic experimental diets were given to the fingerlings twice daily for a period of 84 days. The control diet (FM100) contained 300 g/kg of fishmeal, and the second diet, (BSF50), was formulated to substitute 50% of fishmeal with BSFLM. The fifty percent substitution of fishmeal in BSF50 diet were selected based on previous studies on fishes that successfully substituted fishmeal with 50 percent of dietary BSFLM without adverse effect (Abdel-Tawwab et al. 2020; Muin et al. 2017; Renna et al. 2017). Furthermore, our preliminary study in non-biofloc system also showed that fifty percent of fishmeal could be successfully substituted with BSFLM without hampering the growth performance in terms of

specific growth rate and feed conversion ratio. The nutritional composition of the raw ingredients used to produce the diets, formulated feed and fish body composition were determined by the AOAC (2012) method prior to the formulation of the feed. Both diets contained approximately 33% protein and 10 % lipid, which were formulated using the feed formulation software (Winfeed version 2.8, Cambridge, UK). Winfeed automatically calculated the amount of each ingredient needed to meet the set criteria based on the proximate composition of the raw ingredients. As the BSFLM and fishmeal are different in nutritional composition (Table 1), the other ingredients were adjusted to obtain the isonitrogenous and isolipidic diet condition. The experimental diets formulation and proximate composition are shown in Table 1. As for the commercial diet, the data are limited to the ingredients and proximate composition as the amount were not specified by the producer. The raw ingredients were bought from a local supplier (Nutrivet Sdn. Bhd, Nilai, Malaysia), while the black soldier fly larvae in this experiment were reared in the laboratory. Ingredients were finely grounded (DFY-200C Disk Mill, Orimas, Kuala Lumpur, Malaysia) and sieved before being weighed and mixed following formulation. A mini meat chopper machine (TBS-200 Meat mincer, Orimas, Kuala Lumpur, Malaysia) with a 3 mm diameter die was used to produce slow sinking pressure-pressed pellets, suitable for fingerlings mouth gape size which were then dried in an oven (60 °C) for 24 h before being stored in a cold room (4 °C) until the start of the experiment. The amino acid composition of the fishmeal, black soldier fly larvae meal and the experimental diets are shown in Table 2.

BIOFLOC TANKS PREPARATION

Prior to the experiment, molasses and feed were supplemented to raise the carbon/nitrogen (C/N) ratio of the water column to 10. The calculation of carbon source to be added in the water to create the desired C/N ratio is in accordance to Abu Bakar et al. (2015). The commercial bacteria (BacterGen•F, Continuum Aquatics, Fort Payne, USA) were inoculated to speed up the flocs formation. Nine concrete tanks (270 cm × 210 cm × 70 cm) were supplied with constant aeration and agitation to ensure a high dissolved oxygen (DO) concentration (8403 AZ Dissolved Oxygen Meter, AZ Instrument Corp., Taichung, Taiwan) of around 6 - 7 mg/L. Before starting the feeding trial, the systems were monitored for two weeks to ensure their stability in terms of low TAN, nitrite, and nitrate concentrations.

TABLE 1. Proximate composition of fishmeal, black soldier fly larvae meal (BSFLM) and

Items	Fishmeal	BSFL meal	Treatment		
			Commercial diet ^d	FM100	BSF50
Ingredients					
Fishmeal			(Amount not specified)	300.0	150.0
Black soldier fly larvae powder				0.0	150.0
Soybean meal				243.3	281.6
Rice bran				182.7	184.5
Corn meal				198.5	180.8
Vitamin mixture ^a				10.0	10.0
Mineral mixture ^b				10.0	10.0
DCP				10.0	10.0
Fish Oil				45.6	23.1
Proximate composition (g/kg)					
Crude protein	612.5	485	320.0	330.0	320.0
Crude lipid	92.9	145	50.0	98.2	96.5
Dry matter	951.7	924.7	900.0	915.2	954.4
Ash	252.2	63	100.0	124.7	98.5
Crude fibre			50.0	13.0	21.0
NFE			480.0	435.0	464.0
Gross energy (kJ g ⁻¹) ^c			18.086	19.451	19.655

^aVitamin mixture 100 g⁻¹ diet: vitamin A, 500 IU; vitamin D3, 100 IU; vitamin E, 0.75 mg; vitamin K, 0.02 mg; vitamin B1, 1 mg; vitamin B2, 0.5 mg; vitamin B3, 0.3 mg; vitamin B6, 0.2 mg; vitamin B12, 0.001 mg; vitamin C, 0.1 mg; niacin, 0.2 mg; folic acid, 0.1 mg; biotin, 0.235 mg; panthothenic acid, 1.0 mg; and inositol, 2.5 mg

^bMineral mixture kg⁻¹ diet: selenium, 0.2 mg; iron, 8 mg; manganese 1 mg; zinc, 8 mg; copper, 0.15 mg; potassium chloride, 0.4 mg; magnesium oxide, 0.6 mg; sodium bicarbonate, 1.5 mg; iodine, 1 mg; and cobalt, 0.25 mg

^cas per Schulz et al. (2008)

^dDindings Starter Fish Pellet, Dindings Soya & Multifeeds Sdn. Bhd., Kuala Lumpur, Malaysia: soybean meal, fishmeal, wheat by-products, fish oil, amino acids, vitamins and minerals, antioxidant and mould inhibitor

TABLE 2. Amino acid composition (% as fed) of experimental diets, black soldier fly larvae meal and fishmeal

Amino acid	Diets		Fishmeal	Full fat black soldier fly larvae meal
	FM100	BSF50		
EAAs				
Val	0.68	0.55	0.73	0.89
Leu	1.95	1.57	2.19	1.94
Ile	0.57	0.47	0.61	0.64
Met	0.54	0.45	0.56	0.38
Thr	1.21	0.96	1.04	1.41
Phe	1.16	1.04	1.25	1.19
Arg	0.03	0.03	0.05	0.07
Lys	2.43	1.60	2.33	2.63
His	0.13	0.10	0.18	0.51
NEAAs				
Ala	1.94	1.61	2.24	2.23
Gly	2.47	1.62	3.73	2.30
Asp	1.91	1.36	1.18	1.52
Hyp	0.25	0.07	0.38	0.09
Cys	ND	ND	0.16	0.02
Glu	2.54	2.10	2.08	1.74
Asn	ND	0.14	ND	ND
Gln	1.43	1.44	3.80	1.04
Tyr	1.90	1.93	1.56	2.69
Pro	1.54	1.20	2.30	1.42
Ser	1.45	1.47	2.04	1.77

EAAs, essential amino acid; NEAAs, non-essential amino acids; Val, valine; Leu, leucine; Ile, isoleucine; Met, methionine; Thr, threonine; Phe, phenylalanine; Arg, arginine; Lys, lysine; His, histidine; Ala, alanine; Gly, glycine; Asp, aspartic acid; Hyp, hydroxy proline; Cys, cystine; Glu, glutamic acid; Asn, asparagine; Gln, glutamine; Tyr, tyrosine; Pro, proline; Ser, serine. ND, not detected and is below limit of quantification.

FISH AND REARING CONDITIONS

Upon arrival, one hundred and eighty jade perch fingerlings bought from a local supplier (Diamond Creeks Aquatech Sdn. Bhd., Tanjung Malim, Malaysia) were acclimated for two weeks in the biofloc tanks with commercial feed prior being subjected to three different dietary treatments (BSF50, FM100 & COM (Dindings Soya & Multifeeds Sdn. Bhd., Kuala Lumpur, Malaysia)). All the procedures were performed following Institutional of Animal Care and Use Committee (IACUC) Universiti Malaya guidelines on the usage of animals for experimental study and scientific purposes. The fish were weighed (28 g average weight), placed inside nine tanks (3 tanks/diet, 20 fish/tank) and fed with the experimental diets until apparent satiation daily at 09:00 h and 15:00 h. The amount of the feed given was recorded, and the uneaten feed was collected, dried, and weighed at the end of each feeding to maintain the water quality and for actual feed intake calculation. The water quality, such as the pH, temperature, alkalinity, DO, ammonia, and nitrate concentration, was monitored weekly. The water temperature was maintained at 28 C, DO at 7 mg/L, pH at 7 - 8, NO₃ at 0 - 40 mg/L and NO₂ at 0 mg/L. The photoperiod was natural during the experimental period (12D/12L) with similar lighting conditions for all tanks. Dechlorinated tap water was topped-up as needed to compensate for evaporation loss, with a minimal water exchange rate of 10% once per week. Molasses was supplemented weekly to maintain the C:N ratio based on the total feed.

FISH SAMPLING

At the end of the experiment, the fish were fasted for 24 h before harvested. All individuals in the tanks were weighed and measured for Fulton's condition factor calculation. A total of 10 fish per tank were sacrificed, with five of them used for the sensory characteristic of fillet analysis, and the other five were used for proximate composition analysis of fillet and the calculation of hepatosomatic index (HSI) and visceral fat somatic index (VFSI). For the sensory characteristic of fillet analysis, the fish were washed, skinned, filleted, and vacuum-packed before being stored in -20 °C for sensory analysis. For proximate composition analysis purposes, five fish from each tank were sacrificed, filleted and dissected for liver excision and visceral fat collection for HSI and VFSI calculation, respectively.

GROWTH PERFORMANCE ANALYSIS

The growth performance indicators of the fish included the body weight gain (BWG), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), survival rate (SR) and fillet yield (FY). The formulas for the calculations involved were as follows:

$$\text{BWG (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{FCR} = \text{total feed intake (g)} / \text{weight gain (g)}$$

$$\text{SGR (\%/day)} = [\ln(\text{final weight}) - \ln(\text{initial weight})] / \text{days of feeding} \times 100$$

$$\text{PER} = \text{weight gain (g)} / \text{protein intake (g)}$$

$$\text{SR (\%)} = (\text{final number of fish} / \text{initial number of fish}) \times 100$$

$$\text{FY (\%)} = 100 \times \text{fillet weight} / \text{body weight}$$

BODY INDICES

Body indices such as the Fulton's condition factor (K), hepatosomatic index (HSI), and visceral fat somatic index (VFSI), were calculated according to Du and Turchini (2022) as the following:

$$K = 100 \times \text{body weight} / \text{body length}^3$$

$$\text{HSI (\%)} = 100 \times \text{liver weight} / \text{body weight}$$

$$\text{VFSI (\%)} = 100 \times \text{visceral fat weight} / \text{body weight}$$

NUTRITIONAL COMPOSITION OF FISH AND EXPERIMENTAL DIETS

The proximate analysis of the raw ingredients, experimental pellet, and fish fillet for crude protein, dry matter, moisture content, ash content, and crude lipid was conducted at the Fisheries Research Institute of Glami Lemi, Jelebu, Negeri Sembilan, Malaysia, according to the method of the AOAC (2012). Three replicates were done on each sample. Nitrogen free extract (NFE) were calculated as: 100 - (% crude protein + % crude fat + % crude fibre + % crude ash) while gross energy was measured by using the following factors: crude protein = 23.9 kJ/g, crude lipids = 39.8 kJ/g, and NFE = 17.6 kJ/g (Schulz et al. 2008). The pellets crude fiber and the amino acids composition were determined by external laboratory service using the MS ISO 6865:2003 certification method and in-house GCMS method, respectively.

SENSORY EVALUATION

The study of sensory analysis was performed under experimental conditions at the Biotechnology laboratory in Faculty of Science, Universiti Malaya. A total of five fillet samples from different treatments were evaluated: three from this experimental groups: FM100 (B); BSF50 (B); and COM (B) (cultured in biofloc system) and another two samples from preliminary experimental groups: FM100; and BSF50 (cultured in non-biofloc system). As a preliminary to select the best dietary BSFLM dosage to carry out in the biofloc trials, the jade perch juveniles were

reared without the biofloc system and given diets with the replacement of fishmeal at 0%, 25%, 50%, and 75%. Based on our unpublished data, the group fed BSF50 and FM100 performed similarly in terms of growth and hence are included in this sensory evaluation study to compare their sensory attributes with the biofloc-reared fish. Fifty untrained panelists ($n = 50$) were allocated for this study, consisting of staff and student volunteers. Prior to the panelist selection, several considerations were evaluated. They were non-vegetarian, had no allergenicity to seafood and consumed fish at least twice weekly. Each panelist was briefed about the feed used, fish species, processing and cooking method before the evaluation to ensure they were fully informed about the whole process.

The frozen fillets were thawed to room temperature, cut into similar cuttings ($2 \text{ cm} \times 2 \text{ cm}$), covered with aluminum foil, and steamed (NS628 Food Steamer, Nakada, Kuala Lumpur, Malaysia) for 10 min. The cooked fillets were placed into transparent plastic containers with lids labeled with three random digits labels. The samples were served warm and freshly steamed. Tap water and unsalted crackers were prepared as palate cleansing between tasting samples. The sensory analysis of the steamed cooked fillet of the different treatments was conducted based on the consumer's evaluation and acceptance level. For consumers' evaluation of fillet, these parameters were evaluated: appearance, odour, texture, and juiciness, with the scale ranging from 1 to 8 (score 1, lowest intensity; score 8, highest intensity). The overall fillet evaluation score was calculated by adding all the scores together. The consumer acceptance level survey consists of these parameters: colour, aroma, texture, flavour, and overall evaluation, and scored with the 9-point hedonic scale with numerical ranging between 1 and 9. Both scoring scales are as illustrated in Table 3. Survey forms were provided to panelists as a means of recording the scores.

STATISTICAL ANALYSIS

All data are presented as mean \pm standard deviation. Using the Statistical Package for the Social Sciences (SPSS) 27.0 for Windows (SPSS, Chicago, IL, USA), the experimental data were subjected to one-way analysis of variance (ANOVA), followed by Tukey post-hoc for multiple comparison where there was significant difference ($p < 0.05$). The experimental data were subjected to Welch ANOVA combined with the post hoc Games-Howel when there was non-homogeneity of variances. When a correlation is assumed to exist, the sensory data were subjected to the Pearson's correlation analysis and deemed significant when $p < 0.05$.

RESULTS

GROWTH PERFORMANCE AND BODY COMPOSITION

There were no significant differences in growth performance in terms of body weight gain, feed conversion ratio, specific growth rate, protein efficiency ratio, survival rate and fillet yield across all treatments as indicated in Table 4. The FM100 group obtained the highest body weight gain numerically at $89.10 \pm 1.70 \text{ g}$, followed by BSF50 ($88.45 \pm 2.47 \text{ g}$) and the lowest weight gain obtained by COM ($84.55 \pm 12.30 \text{ g}$). The same trend is observed in terms of specific growth rate, with FM100 obtaining the highest daily growth ($1.81 \pm 0.02 \text{ \%/day}$) followed by BSF50 ($1.80 \pm 0.03 \text{ \%/day}$) and COM ($1.71 \pm 0.08 \text{ \%/day}$). Meanwhile, BSF50 has been observed to show the best values in FCR (1.77 ± 0.04), PER (1.77 ± 0.04), and FY ($37.08 \pm 8.42 \text{ \%$) numerically. The survival rate was above 90% in all the treatments. For fillet proximate composition, all groups showed no significant differences in terms of crude protein, and moisture as illustrated in Figure 1. The lipid content, however, was significantly lower in the FM100 group ($16.50 \pm 1.73 \text{ g/kg}$), while the BSF50 and the commercial group have no significant difference.

BODY INDICES

The hepatosomatic index indicator as presented in Figure 2 shows that there was a significant difference between FM100 group (1.70 ± 0.42) with BSF50 group (3.13 ± 0.25), while there is no significant difference between FM100 and COM group. The visceral fat somatic index differs significantly between all groups, with BSF50 group obtained the highest value (10.89 ± 1.6). Fulton's condition factor on the other hand is not significantly different between the groups.

SENSORY EVALUATION

The sensory evaluation involved three samples from the current trial in the biofloc system: FM100 (B); BSF50 (B); and COM (B) and two samples from the preliminary trial in the non-biofloc system: FM100; and BSF50. The results are illustrated in Figure 3. Based on the consumers' evaluation, the brightness of fillet was significantly different among COM (B), and FM100 ($p < 0.05$). Fillet cultured in a non-biofloc system and given the control diet (FM100) have the brightest appearance (6.24 ± 1.36) followed by those in the same system given BSF50 diet (5.78 ± 0.91). The COM (B) group had the darkest appearance among all the treatments (4.26 ± 1.29). The odour was most intense in COM (B) group (5.96 ± 1.29).

TABLE 3. The scoring used for consumer sensory evaluation and acceptance level of cooked jade perch fillet

8-Point scoring									
Score	1	2	3	4	5	6	7	8	
Brightness	Extremely Dark	Very dark	Moderately dark	Slightly dark	Slightly light	Moderately light	Very light	Extremely light	
Odour	Extremely weak	Very ¹ weak	Moderately weak	Slightly weak	Slightly strong	Moderately strong	Very strong	Extremely strong	
Tenderness	Extremely tough	Very tough	Moderately tough	Slightly tough	Slightly tender	Moderately tender	Very tender	Extremely tender	
Juiciness	Extremely dry	Very dry	Moderately dry	Slightly dry	Slightly juicy	Moderately juicy	Very juicy	Extremely juicy	
9-Point Hedonic Scale ¹									
Score	1	2	3	4	5	6	7	8	9
	Extremely Dislike	Dislike Much	Moderately Dislike	Slightly Dislike	Neither Like nor Dislike	Slightly Like	Moderately Like	Like Very Much	Extremely Like

¹Hedonic scale labelling in accordance to Kalva et al. (2014) and Peryam and Pilgrim (1957)

TABLE 4. Effects of different dietary treatments on growth performance parameters of jade perch

Group	FM100	BSF50	COM	P-value
Growth performance				
BWG, g	89.10 ± 1.70	88.45 ± 2.47	84.55 ± 12.30	0.725
FCR	1.78 ± 0.01	1.77 ± 0.04	1.87 ± 0.22	0.575
SGR, %/day	1.81 ± 0.02	1.80 ± 0.03	1.71 ± 0.08	0.107
PER	1.71 ± 0.01	1.77 ± 0.04	1.69 ± 0.20	0.693
SR, %	98.48 ± 2.14	90.00 ± 4.71	96.67 ± 4.71	0.235
FY, %	34.94 ± 3.47	37.08 ± 8.42	36.40 ± 8.19	0.789

BWG, body weight gain; FCR, feed conversion ratio; SGR, specific growth rate; PER, protein efficiency ratio; SR, survival rate; FY, fillet yield. Dietary treatments; FM100 = control diet; BSF50 = substitution of fishmeal with 50% BSF meal; COM= commercial diets. Data were subjected to one-way analysis of variance (ANOVA)

while there were no significant differences between all other groups. The BSF50 group (4.14 ± 1.20) was the least moist and least tender compared to the other groups. The overall score indicated a significant difference between BSF50 and COM (B) fillet. For the consumers' acceptance of fillet, in terms of colour, the FM100 scored the highest acceptance (6.6 ± 1.37), with the least acceptance observed in COM (B) (5.64 ± 1.52). There were no significant differences in the aroma of fillet between samples ($p > 0.05$). In terms of texture, the FM100 (B) and COM (B) gained comparable results which was the highest level of acceptance, while they are significantly different from those of BSF50 (5.38 ± 1.65). The most flavorful fillet was observed from the FM100 (B) group (6.08 ± 1.46), while the least flavorful was from the BSF50 group. Overall appreciation shows that the FM100 (B) shows the highest score, while it is not significantly different from the COM (B), BSF50 (B), and FM100. BSF50 group which was cultured in the non-biofloc system scored the least (5.14 ± 1.51).

DISCUSSION

To the best of our knowledge, the current study is the first trial on black soldier fly larvae meal in the diets of jade perch cultured in the biofloc system. Growth performance is one of the most critical parameters to observe if the test

diets hamper fish development. In the current study, no significant difference were observed between treatments in FM100, BSF50, and COM in terms of growth indicators which is similar when dietary BSF were included as 50% fishmeal substitution in other species like the rainbow trout (Renna et al. 2017), European seabass (Abdel-Tawwab et al. 2020), and Nile tilapia (Muin et al. 2017). However, a study on meagre found that BSFLM linearly decreased the growth, feed efficiency, and protein efficiency ratio with the increasing fishmeal substitution by up to 52% while not affecting feed intake (Guerreiro et al. 2020). Contrary to these findings, a full-fat BSFLM inclusion of up to 30% in the diet increased the specific growth rate, the body weight and improved the feed conversion ratio and protein efficiency ratio of Siberian sturgeon (Rawski et al. 2020). Similarly, a positive effect on SGR and FCR of juvenile mirror carp was observed when oil derived from BSF was supplemented compared to other insect-based oils (Xu et al. 2020). Both positive and negative outcomes reported in previous studies indicate that tolerance to dietary BSFLM is species specific.

Feed that contributes to low feed conversion ratio (FCR) is sought after in the aquaculture sector to ensure the feed items not only effectively promotes weight gain of farmed fish but are also economical eventually. While there is no significant difference between the treatments, the FCR value in the current study is better compared to jade perch reared on diets constituting various compositions

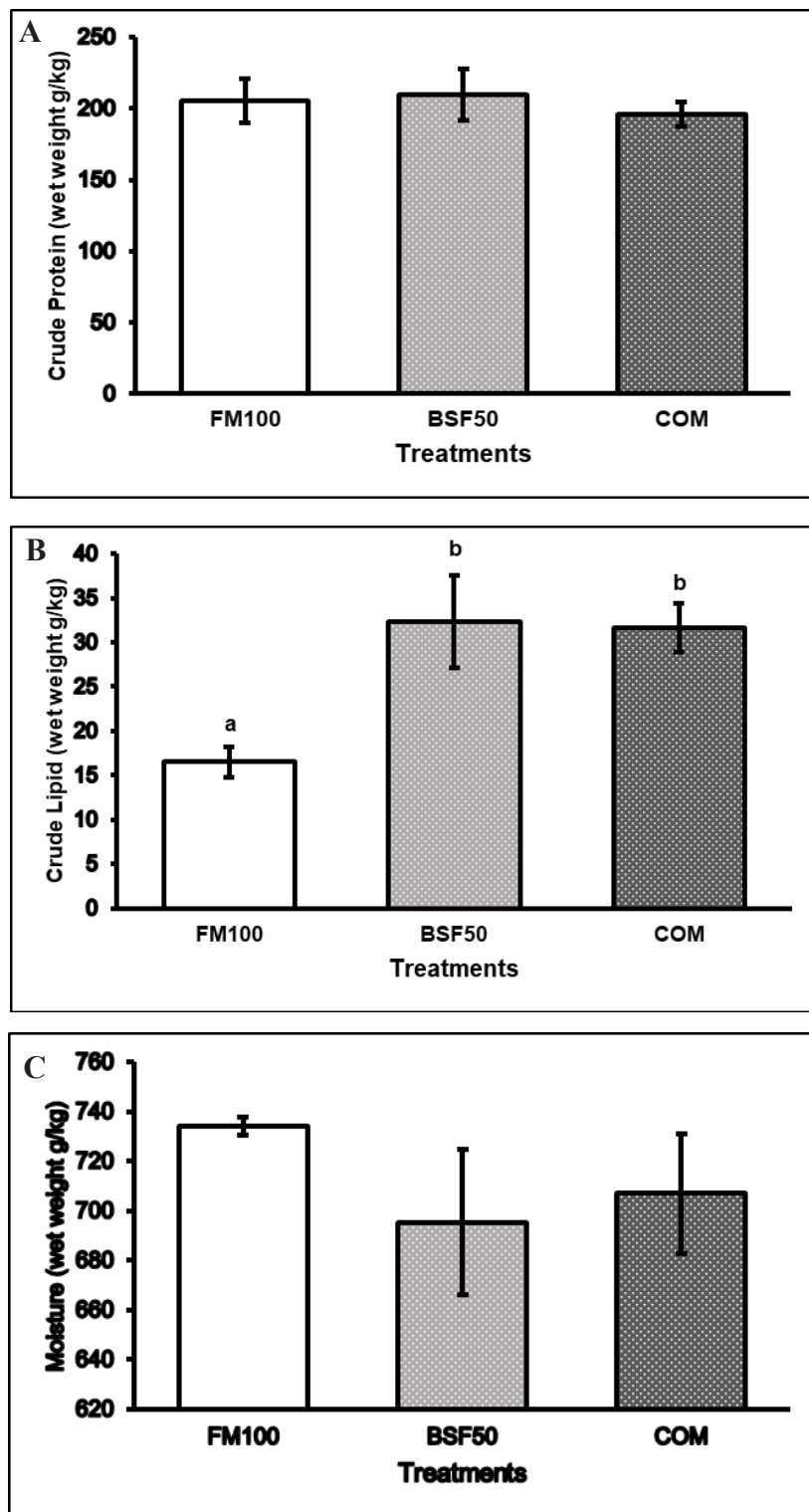


FIGURE 1. Proximate composition of fillet: (A) crude protein, (CP, g/kg); (B) crude lipid, (CL, g/kg); and (C) moisture (g/kg)

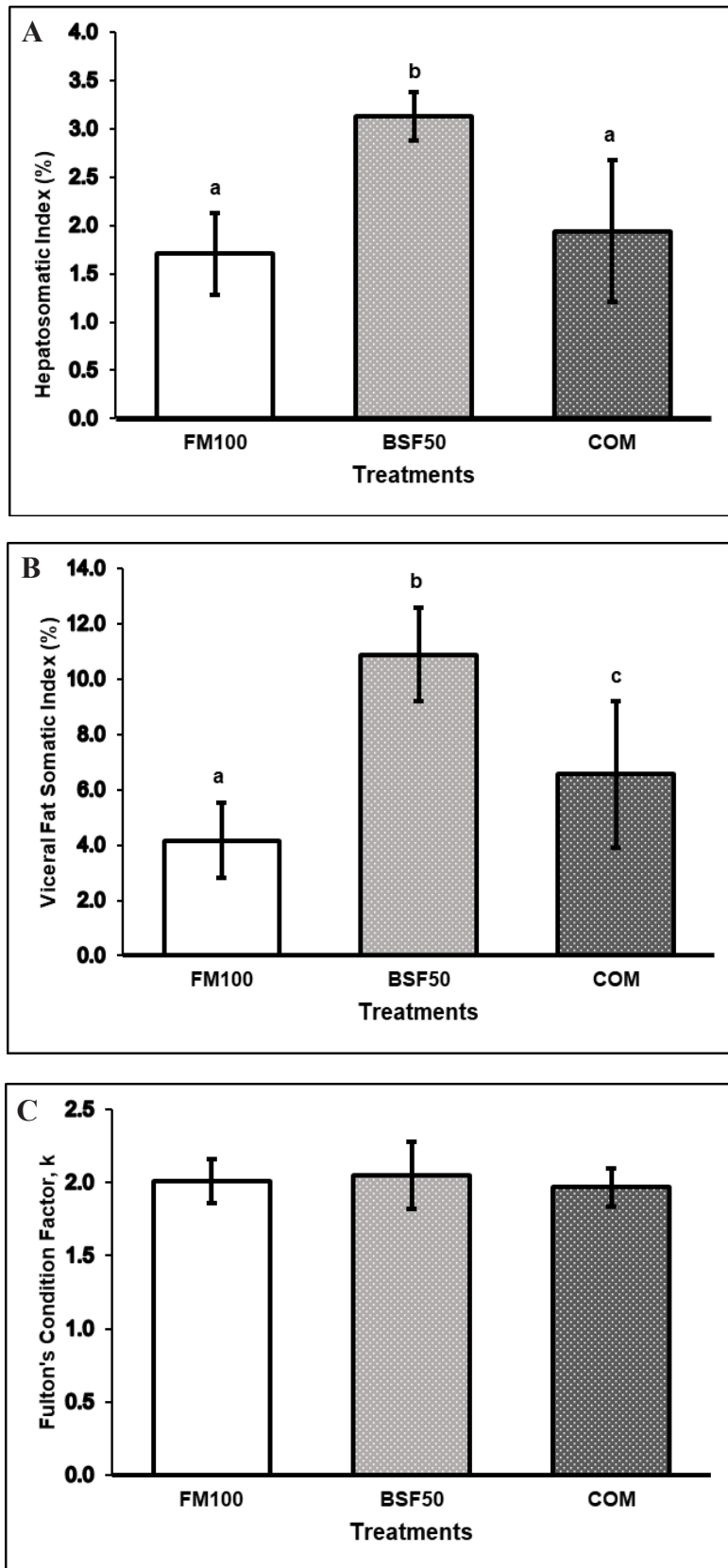


FIGURE 2. The result of (A) hepatosomatic index (HSI, %); (B) visceral fat somatic Index (VFSI, %); and (C) Fulton's condition factor (k) across treatment groups

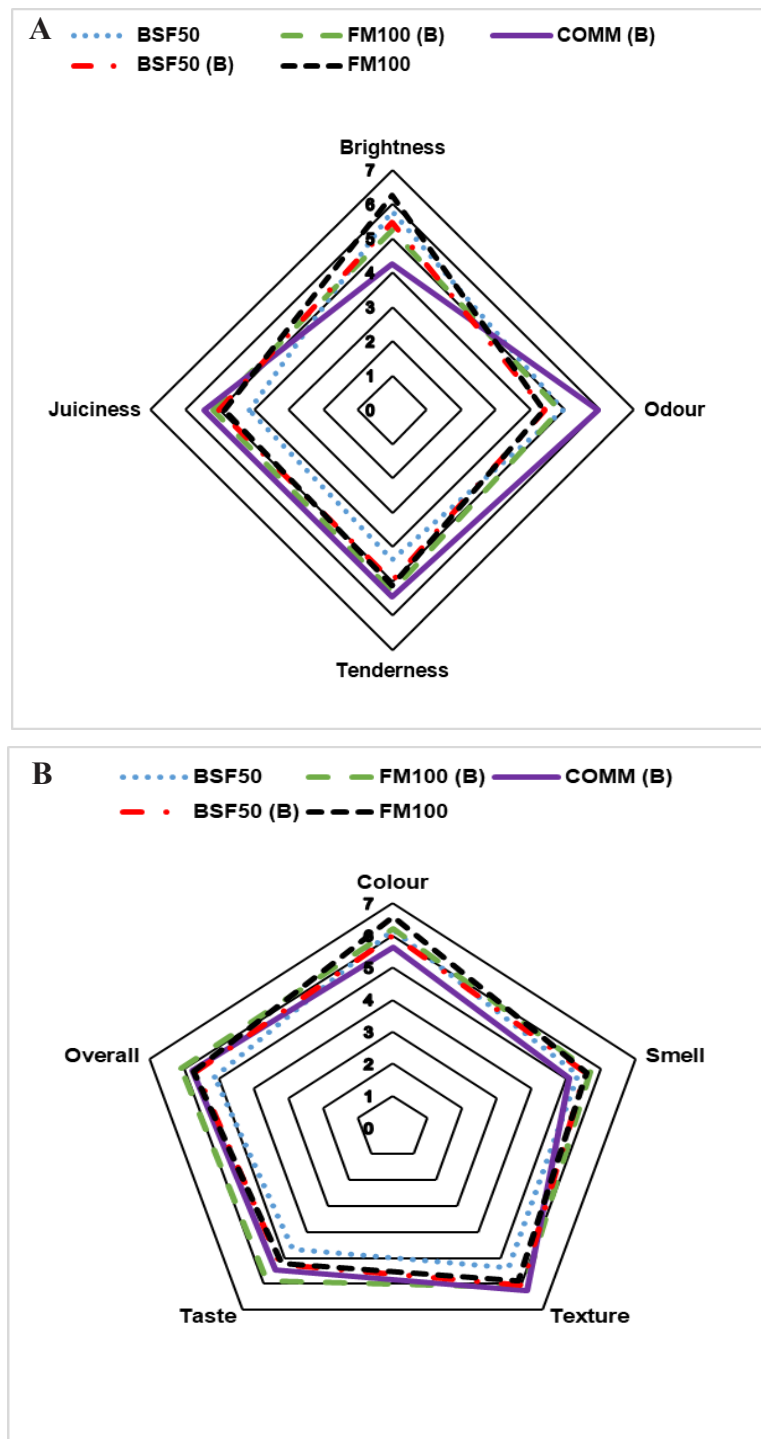


FIGURE 3. Radar charts of the sensory analysis by consumers on (A) perceived characteristic and (B) acceptance level of fillets of jade perch from different treatments namely: FM100 (B) (fed control diet with no fishmeal substitution and cultured in biofloc system); BSF50 (B) (fed experimental diet containing 50% fishmeal substitution with BSFLM and cultured in biofloc system); COM (B) (fed commercial diet and cultured in biofloc system); FM100 (fed control diet with no fishmeal substitution and cultured in non-biofloc system); and BSF50 (fed experimental diet containing 50% fishmeal substitution with BSFLM and cultured in non-biofloc system)

of fermented food waste (1.93 - 2.47) in an experiment by Mo et al. (2019). However, it showed a higher FCR value when compared with the fish group fed twice daily with commercial diet in a recirculating aquaculture system (RAS) carried out by Al-Khafaji et al. (2017). The different FCR outcomes from the studies on this same species might be due to the varied sizes of fish used during respective experiments, the rearing period, or the distinct types of feed given. In the aquaculture production, FCR is a targeted trait for improvement (Fu & Yuna 2022). However, according to Besson et al. (2020), fish breeders often neglect FCR and become fixated on improving the growth rate traits to increase profits, aiming to have maximum production in a short time when, in fact, reducing FCR is an approach that increases profits and reduces environmental impact simultaneously. Moreover, in addition to the reduced amount of feed needed to be purchased for fish to achieve marketable sizes, lower FCR indicates less waste, which requires minor aeration and emits low carbon dioxide from the energy consumption of aerators and oxidation of organic waste (Boyd & Mcnevin 2023). Ranging between 34 and 37%, the fillet yield in this study is comparable to those reported by Van Hoestenberghé et al. (2016) when fish oil was substituted with *Schizochytrium* sp. meal and linseed oil in fishmeal-free diets. However, contrary to the current finding, their findings show significant differences between groups fed fish oil and *Schizochytrium* sp. meal. Ranging from 1.97 to 2.05, the condition factor in the current study is similar between all treatments and close to the values obtained by Al-Khafaji et al. (2017). On the other hand, the increase in hepatosomatic index in BSF50 group is different from a study in brown trout which decreases with BSFL meal inclusion which is assumed to cause a lower fat accumulation in the liver and is considered as a positive effect (Mikołajczak et al. 2022).

In terms of body composition, the crude lipid content of BSF50 and COM groups was not statistically different at 32.33 ± 5.27 g/kg and 31.67 ± 2.76 g/kg, respectively, and higher compared to FM100 (16.5 ± 1.73 g/kg). However, the crude protein and moisture content are not affected by the inclusion of BSFLM ($p > 0.05$). These findings are in agreement to the increase in lipids in the fillet of zebrafish with increasing dietary BSFLM inclusion, with no differences in the crude protein content (Zarantoniello et al. 2020). Certain inclusion of dietary BSFLM in other species, such as the Atlantic salmon (Belghit et al. 2019), rainbow trout (Borgogno et al. 2017), and the European seabass (Abdel-Tawwab et al. 2020), have reported non-significant effect on fillet protein and lipid. The non-significant differences in crude protein content in all treatment groups in the above-mentioned studies indicates that BSF is a comparable protein provider to fishmeal. On another note, while there were no significant differences in crude protein and lipids of

rainbow trout fed dietary BSFLM, differences in the fatty acid profile of fillet were observed (Mancini et al. 2018). Likewise, jade perch's fatty acid composition of fillet also reflected the dietary fatty acid profile when vegetable oils such as canola oil, linseed oil, and sunflower oil substituted the dietary fish oil (Van Hoestenberghé et al. 2013). Fishmeal substitution with up to 45 % with BSFLM in European seabass did not alter their lipid composition (Moutinho et al. 2021). Moreover, while the diets' saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) increased and decreased, respectively, to the increasing BSFLM dietary inclusion, no changes of fatty acid profiles are displayed in the fillet. In the current study, the lipid content of BSFLM is higher than fishmeal which might be the reason for the increase in fillet lipid composition. On top of that, the BSFLM's nutritional content is highly dependent on its substrate composition. For instance, Muin et al. (2022) found that dried BSF larvae reared on soybean curd residue have significantly higher protein ($\sim 46.37\%$) and lower lipid ($\sim 22.02\%$) as opposed to those reared on desiccated coconut which were $\sim 20.07\%$ and $\sim 67.24\%$, respectively. Fifty percent fishmeal replacement with BSFL reared on either coconut pulp (CP) or palm oil decanter cake (DC) in the red tilapia diet, have resulted the DC group to obtain improved growth and feed efficiency, with no significant difference with control fillet compositions of crude protein, lipid, and ash (Taufek, Lim & Bakar 2021). Barroso et al. (2014) also stated that insect meals generally have a higher lipid content compared to fishmeal and soybean meal. Hence, to fit them into the feed formulation, defatting process are commonly employed. Another alternative to improve the lipid profile of BSFLM prior to being used as feed ingredients includes the addition of PUFA-rich ingredients in their substrate as done by Zarantoniello et al. (2020).

The crude protein content of jade perch's fillet obtained in all groups from the current study is higher (19.6 - 20.9 %) than the findings by Al-Khafaji et al. (2017), (15.98 ± 0.32 %) with the same feeding regime but different culturing system. On the other hand, the crude lipid showed a higher value in the previous study at 12.13% but similar moisture content as our finding (67.88 - 69.39 %). A complete substitution of fish oil with *Schizochytrium* sp. meal and linseed oil in a fishmeal-free diet had not influenced the jade perch's muscle composition of moisture (74.14 - 74.79 %), crude protein (19.18 - 19.62 %), and crude lipid (4.87 - 5.78 %) (Van Hoestenberghé et al. 2016). While the protein content is comparable to the current study, the slightly higher lipid in the said study might be due to the high lipid content of the test ingredient, the *Schizochytrium* sp. meal compared to BSFLM.

The versatile dietary amino acids play many functions in fish which include; survival, growth, and muscle development; release of hormones; attractants; immune

responses; lipid digestion and metabolism; and spawning and larval development (Li, Zheng & Wu 2021). The amino acid content in insect meals depends on the rearing substrates, stages of life, species, and processing methods. Barroso et al. (2014) reported that insects of the Diptera taxon showed higher histidine, lysine, and threonine than the other taxa and are the most similar to fishmeal in overall essential amino acids (EAAs) and limiting amino acids. The BSFLM used in this study has slightly higher EAAs of valine, isoleucine, threonine, arginine, lysine, and histidine compared to fishmeal. On the other hand, leucine, methionine, and phenylalanine are higher in the fishmeal with little difference. The current finding is similar in the concentration range for threonine, phenylalanine, lysine, alanine, and glycine obtained in the BSFLM by Abdel-Tawwab et al. (2020). Renna et al. (2017) reported a higher concentration of all amino acids. However, in their study, partially defatted BSFLM, which contains a higher protein and lower lipid content were used. The current study finding is also similar in the higher concentration of valine and histidine than the fishmeal as those reported by Tschirner and Simon (2015).

Bioflocs technology (BFT) have been reported to improve water quality and the growth performance of common carp without adverse effect on its body composition when carbon to nitrogen ratio of 19:1 was employed in a molasses-based biofloc (Minabi et al. 2020). Another study in Nile tilapia reported that tapioca flour as a carbon source in biofloc performed better than the other treatments (control and sugarcane molasses) in terms of fish growth, hematology, immunity, and antioxidant status (Rind et al. 2023). Hence, the selection of the carbon source also plays a significant role in the well-being of the farmed fish. Meanwhile, in the current study that tested different diets in biofloc system, the BSF50 group achieved comparable growth performance with the control and commercial diets ($p > 0.05$). Moreover, the sensory attributes, and consumer acceptance of jade perch fillet fed the insect diet also improved significantly in the biofloc system. In addition to the optimum growth performance of the aquaculture species, sensory evaluation is crucial before the final products enter the market. Hence this study utilized the 9-point hedonic scale, the most common internationally accepted scale to measure the degree of acceptance by the consumer (Navarro et al. 2013). In our findings, the COM (B) fillet was perceived as the darkest in appearance, while FM100 is the brightest. Further, Pearson's correlation analysis showed a positive correlation between the perceived brightness of jade perch's fillet and the consumer level of acceptance ($p < 0.05$). Even though the odour is the strongest in COM (B), it does not affect consumers' odour preferences. The lowest score obtained by the BSF50 group in terms of juiciness and tenderness

perception might influence the group's overall hedonic scores, which is also the least (5.14 ± 1.51). On the other hand, its counterpart, the BSF50 (B), which was cultured in the biofloc, is similar to FM100 (B) and COM (B) concerning texture, taste, and overall appreciation, indicating that the characteristic of the fish fed the same fed stuff can be utterly changed by adjusting the culturing medium.

Meanwhile, another study by Van Hoestenbergh et al. (2016) indicates that the slight change in the feeding period of special feedstuff also changed fillet acceptance. The author reported that feeding the dietary *Schizochytrium* sp. throughout the 14 weeks trial increased the n-3 HUFA in the flesh of jade perch given fishmeal and fish oil-free diet, but this was at the expense of the consumers' preference. On that account, the most viable option was to feed *Schizochytrium* sp. diet only for the additional four weeks after ten weeks of feeding with dietary vegetable oil. This resulted in not only enriched n-3 HUFA but also increased sensory scores such as the flavour, overall appreciation, and purchase intent to be similar to that of fish oil-fed fish.

Although the effect of BSFLM on growth has been extensively reported in various fish species (Prakoso et al. 2022), there are currently limited studies on the sensory evaluation of fishes when fed with BSFLM. Most reported findings were on the salmonids (Belghit et al. 2019; Borgogno et al. 2017; Lock, Arsiwalla & Waagbø 2016; Sealey et al. 2011), with two studies on the freshwater fish fillet evaluation when fed dietary BSFL (Muin et al. 2023; Ordoñez et al. 2022). When Atlantic salmon were fed the BSFLM, complete fishmeal substitution did not significantly affect the sensory evaluation. However, a slight difference in the baked fillet in terms of colour intensity and rancid odour of fish flesh was observed (Belghit et al. 2019). A study by Sealey et al. (2011) on rainbow trout fed fish offal-enriched BSF also reported non-significant differences with the control in a blind comparison sensory analysis. Ordoñez et al. (2022) reported that whole BSFL fed to tambaqui improves the fillet's flavour, odour, and appearance. Furthermore, given a BSFL-based diet with *Gracilaria changii* at 15% fishmeal replacement, red hybrid tilapia gained the preferable attributes regarding flavour and overall acceptability (Muin et al. 2023). When lesser mealworm was used at up to 75% to replace FM and combined with poultry by-product meal in the European perch diet, the fillet sensory had similar odour, flavour, and texture as control along with positive outcomes on the growth and health status of fish. However, the study suggested that 50% replacement with the mealworm can be more advantageous regarding environmental impacts (Tran et al. 2024). Certain free amino acids in the flesh, such as Glu, Asp, Ala, and Gly, contribute to the flavour

and taste of cooked fish (Ruiz-Capillas & Moral 2001; Yang et al. 2022). Therefore, Yang et al. (2022) reported that the high sensory score of flavour was ascribed to the high 'delicious amino acid' concentration found in the largemouth bass flesh in their study. In addition to having a lower steaming loss and drop loss after 6 h, the preferred fish flesh was also characterized by more robust flesh sweetness and freshness, a more elastic texture, and a less fishy taste. The author highlighted that water losses might also cause the loss of soluble amino acids, affecting the quality of flesh. Even though the current study did not conduct the amino acid analysis and the water holding capacity test on fillets, the possibility of these factors' influence on the sensory scores should not be overlooked as steamed fillets from the non-biofloc BSF50 group which scored the lowest for juiciness also scored poorly in terms of taste acceptance.

CONCLUSION

In conclusion, the BSF50 group achieved similar growth performance with the FM100 and COM when cultured in the biofloc system. In addition, the sensory characteristics of BSF50 fed fish are also improved when cultured in the biofloc system. However, there's a slightly higher fat accumulation in terms of crude lipid content, HSI and VFSI compared to the other treatments. As the BSFLM in this study has a higher lipid content compared to fishmeal, the fatty acids constituents might also be different, leading to different fatty acid metabolism pathways. Further exploration of fatty acid and amino acid composition in jade perch fed dietary BSFLM in biofloc culture might shed light on its nutritional contribution.

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