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RESEARCH ARTICLE

Use of black soldier fly (*Hermetia illucens*) prepupae reared on organic waste as feed or as an ingredient in a pellet-feed formulation for Nile tilapia (*Oreochromis niloticus*)

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Abstract

The growth and sustainability of freshwater aquaculture are highly dependent on economic feed which is the major running cost. Fish feed industries depend on the high-priced fish meal (FM) as protein source in feed formulations. In this context, a nutrient-rich, and palatable insect meal–based fish feed was developed incorporating the black soldier fly (BSF) (*Hermetia illucens*) prepupae meal (BSFPM) reared on organic waste imparting additional benefit of waste bioremediation to make cost-effective feed. Feeding trial was conducted to evaluate growth performance on monosex Nile tilapia (*Oreochromis niloticus*). The different treatments were (1) dried BSF prepupae, (2) BSF prepupae and BSFPM-based feed in 1:1 proportion, (3) BSFPM feed, and (4) control feed with FM. The survival, growth, feed efficiency, and haematological parameters were not significantly different between BSFPM and control feed. Fish fed with control feed and BSFPM feed showed significantly higher ($P \leq 0.05$) weight gain, specific growth rate, and percentage weight gain. Lowest food conversion ratio ($P \leq 0.05$) was recorded for fish fed control feed with a significantly higher feed efficiency ratio ($0.65^{d} \pm 0.034$) and protein efficiency ratio ($2.11^{a} \pm 0.063$). The mean corpuscular volume of blood in fish fed BSF prepupae ($128.5^{a} \pm 3.2$) is significantly higher. The good growth of fish fed BSFPM feed may be attributed to the essential amino acids which are not limiting in feed. Absence of microbes and safe level heavy metals in BSFPM feed ensures safety of the ingredient. Hence, it can be used as a suitable protein source in feed formulations.

Keywords Black soldier fly · Waste · Formulated feed · Proximate composition · Tilapia · Growth · Haematology

Introduction

The development of the aquaculture sector is highly dependent on the nutritionally balanced fish feed which is a major recurring cost. The major protein component used in fish feed formulations is the fish meal and the aquaculture profitability is affected by the high cost of fish meal. Overexploitation of fish stocks for fish meal affects the sustainability of these resources in the wild. Hence, an alternative ingredient to replace fish meal in aquaculture feed which is economically and environmentally viable is of primary interest worldwide. Insects can be a good alternative as a feed ingredient as they can be reared on organic wastes by sustainable and cost-effective farming methods (Meneguz et al. 2018). The possibility and potential of the exploitation of insects as an animal feed ingredient are being exploited worldwide with promising results (Borgogno et al. 2017; Dumas et al. 2018; Vargas et al. 2018). During the last few years, there have been investigations on the efficacy of insect meal in the formulation of fish feed for several cultivable freshwater fish species. Among several potential insect species which can be utilised as animal

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feed, particularly the black soldier fly (BSF) (*Hermetia illucens*) Linnaeus 1758 (Diptera: Stratiomyidae) gained most attention on a commercial scale (Lock et al. 2015) for being a potential source of protein in feed formulations (Cammack and Tomberlin 2017).

The BSF can be cultivated on organic wastes such as agricultural by-products, animal manure (Diener et al. 2011), waste from fin fishes, and shell fishes (Sealey et al. 2011; Villazana and Alyokhin 2019). They convert these wastes to quality protein (Newton et al. 1977; Wang and Shelomi 2017), which consequently act as a means of bioremediation. The BSF can also be reared on home-based food wastes which are usually generated in large quantities in urban areas and are potential environmental pollutants causing human health hazards (Li et al. 2011). Thus, the rearing of BSF larvae is an eco-friendly waste management option (Warburton and Hallman 2002). BSF is reported to breed prolifically in organic wastes, producing protein rich biomass utilising 50% of manure waste (Sheppard et al. 1994). Bio-waste degradation using biological means is an efficient way of waste disposal (Diener et al. 2009) and BSF control the growth of pathogenic bacteria like *Salmonella* sp. and *Escherichia coli* during organic waste decay (Lalander et al. 2013).

Considering the nutritional quality of BSFL, it is rich in protein (Cummins et al. 2017), lipids (Li et al. 2016), minerals (Spranghers et al. 2017), and contains chitin (Caligiani et al. 2018). In addition, BSFL possesses an essential amino acid profile, which is comparable to the amino acid profile of fish meal, rendering it most suitable as a fish feed. But they remain rich in saturated fatty acids compared to polyunsaturated fatty acids unlike fish (Barragan-Fonseca et al. 2017). They also have high ash as well as crude protein content than many insects that are used as feed ingredients (Barroso et al. 2014). Hence, to characterise the potentiality of BSFL as a feed and feed ingredient, feeding trials were conducted to evaluate the growth of monosex Nile tilapia on different feed forms of dried BSF pre-pupae with a control excluding BSFL. The potential utilisation of BSFL meal in fish feeds could reduce feed manufacturers' and fish farmers' dependency on fishmeal as an alternate protein source which could eventually lead fish farming into an economically and environmentally viable venture. Therefore, the present study evaluated the effect of replacement of fishmeal with BSFL meal on feed efficiency, growth rates, serum biochemistry, and haematology in juvenile Nile tilapia. Haematological and serum biochemical parameters of the fish are indicators to monitor the nutritional effects and health status of the fish. These parameters were evaluated to understand the stress related to nutrition if any. The tilapia fish in particular was selected for evaluating different experimental feeds containing BSF prepupae as it is a widely cultured fish and has an omnivorous feeding habit that accepts a wide selection of food.

Materials and methods

The experiment was conducted following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 2011). The feeding trial was carried out for a period of 90 days to evaluate the effect of BSFL on the growth performance of tilapia. The experimental fish were procured from a government hatchery in Kerala, India. The experimental setup consisted of 12 glass tanks of 172-L water capacity. During the trial, the temperature averaged 25 ± 2 °C, dissolved oxygen was maintained above 5 mg L^{-1}, and nitrogenous compounds were below 0.02 mgL^{-1}.

The experiment was based on a completely randomised design comprising four treatments with three replicates. The different treatments are detailed in Table 1. Prior to the start of the experiment, the fish were acclimatised for 2 weeks. Fish of average weight (3.98 ± 0.07 g) and length (6.3 ± 0.26 cm) were stocked at the rate of 150 $no.m^{-3}$. The feeding rate was maintained at 5% of body weight per day. The daily ration was divided into two equal doses a day offered at 09:00 and 16:00 h. Water exchange was carried out once in 2 weeks to ensure the water quality parameters to be in the optimum range for fish growth. Faecal matter accumulated was collected daily before the morning feed. At the termination of the experiment, fish were weighed individually taking representative samples from each tank following 1 day of feed deprivation. The length and weight of the fish were measured every 2 weeks and the amount of feed provided to the fish was adjusted accordingly.

BSF rearing and prepupae production

BSF was reared at ICAR–National Bureau of Agricultural Insect Resources (NBAIR), Bangalore (77°34′ E; 13°54′ N) using organic wastes. For the rearing, equal proportions (500 g each) of vegetable and fruit wastes collected from the local markets were stored in a rearing box (20 × 20 cm)

Table 1 The treatments based on different experimental feeds

Treatments	Feed
T1	100% dried BSF prepupae
T2	50% dried BSF prepupae + 50% formulated feed containing BSF prepupae meal
T3	100% formulated feed containing BSF prepupae meal
Control	100% formulated feed containing fish meal

in the laboratory (60 ± 10% RH, 27 ± 2 °C temperature, 60–70% moisture). About a hundred numbers of 1-day-old BSF larvae were inoculated over the rearing substrate in plastic rearing boxes covered with a black coloured muslin cloth. The substrate was monitored on daily basis for feeding of the larvae till pupation. After completion of the larval stage, the prepupae were manually harvested from the substrate. The harvested prepupae after thorough washing was killed by keeping in refrigerator for 1 day. The dead prepupae was then sun dried for 7 days and then used for feed preparation (Panikkar et al. 2018a). The dried BSF prepupae with a crude protein content of 32.53% and crude lipid of 22.1% was ground to make powder before incorporating in feed. The prepupae development and feeding are graphically represented in Fig. 1.

Analysis of chitin content in dried BSF prepupae

The chitin content in BSF prepupae was analyzed according to Caligiani et al. (2018). The BSF prepupae dried to a constant weight was ground to powder using a mixer. One-molar HCl was added to the powder for demineralisation at an hour interval for 6 h. The demineralised powder was then washed with distilled water, followed by de-proteinisation using 1 M NaOH keeping at 80 °C for 24 h. The de-proteinised extract was then filtered and de-coloured using 1% $KMnO_4$. The de-coloured filtrate was washed with distilled water and dried to constant weight before weighing.

Analysis of amino acid profile of dried BSF prepupae and BSFL diet

The amino acid composition was determined by using high-performance liquid chromatography (HPLC) (1525, Waters) equipped with a C18 reverse-phase column (WAT052885) and a fluorescence detector (2475, Waters) following Ishida et al. (1981) and Sastry and Tammuru (1985).

Analysis of the fatty acid profile of dried BSF prepupae

Fatty acid compositions of the sample were determined by gas chromatography–mass spectrometry (GC–MS). The identification and quantification were done using a GC (Trace GC Ultra, Thermo Scientific) equipped with a capillary column (TR-FAME, 30 m × 0.25 mm, 0.25 µm film thickness) and an MS (ITQ 900, Thermo Scientific) attached to it following the procedure described by Folch et al. (1957), Metcalfe et al. (1966), and Mohanty et al. (2013)

Fig. 1 BSF prepupae development and feeding of *O. niloticus*

Microbial analysis of BSFL

The microbial analysis of BSF prepupae was carried out. The dried BSF prepupae was ground to powder and 25 g of this powder was added to phosphate buffer to make a 250-mL mixture by blending in a stomacher for homogenous mixing. Aliquots of different dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}) were prepared using phosphate buffer for enumeration. The presence of *Salmonella* and *Escherichia coli* was analysed based on the standard biochemical procedures (Alfrad 2007; Marjan et al. 2014).

Analysis of heavy metals in BSFPM-based feed

The possibility of occurrence of heavy metal in BSFL-incorporated feed was analysed as the BSFL was reared on vegetable waste. The analysis was carried out to estimate the presence of arsenic, cadmium, chromium, lead, nickel, and mercury in the formulated feed. The feed samples were digested (Al-Weher 2008) and analysed for heavy metals using atomic absorption spectrophotometer, and the recovery for each metal was calculated (Uba et al. 2008).

Biochemical analysis of BSFL-incorporated feed and experimental fish

Biochemical analysis of feed and fish was done to estimate the proximate composition following standard methods of AOAC (1995). Moisture, crude protein, ether extract, ash, and total carbohydrate composition of BSFPM feed and fish after the 90 days of feeding trial were estimated. The fish were individually weighed and dried for proximate composition estimation at the termination of the experiment.

Growth parameters of O. niloticus

On the termination of the feeding trial, the fish were counted and weighed to estimate various parameters such as percentage weight gain, specific growth rate (SGR), feed efficiency ratio (FER), protein efficiency ratio (PER), and food conversion ratio (FCR). Feeding was suspended for a day prior to taking the body weight measurement. The growth performance indices were calculated using the following formulae:

$$\text{Weight gain (g)} = \text{mean initial body weight (g)} - \text{mean final body weight (g)}$$

$$\text{SGR} = 100 \times (\ln \text{final body weight (g)} - \ln \text{initial body weight (g)})/\text{days of trial}$$

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

$$\text{FER} = \text{wet weight gain (g)}/\text{dry feed intake (g)}$$

$$\text{PER} = \text{wet weight gain (g)}/\text{crude protein fed (g)}$$

Effect of BSFL-based diet on haematology of *O. niloticus*

The effect of BSF prepupae-based diet on the haematology of *O. niloticus* was evaluated based on the parameters such as haemoglobin (Hb) content, haematocrit (HCT) value, red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC). Blood samples were collected from the caudal vein of the fish, using a 2-mL sterile disposable plastic syringe (23 G) coated with anticoagulant (10% EDTA). The blood was then transferred into vials containing EDTA to avoid clotting and was analysed immediately after collection. The total RBC and WBC counts were estimated using an improved Neubauertype haemocytometer (Pradhan et al. 2014; Guijarro et al. 2003). The number of RBCs/WBCs was expressed as cells per cubic millimetre and was calculated as follows:

$$\text{Total number of RBCs/WBCs} = \frac{\text{Number of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{Area counted}}$$

The haemoglobin (Hb) content was estimated by acid–haematin method using Sahli's haemocytometer. The amount of haemoglobin was directly read in g/dL. The haematocrit (Hct) was determined by using microhaematocrit reader and the values were expressed in % (Wintrobe 1974). The erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the respective formula (Dacie and Lewis 1994):

$$\text{MCV } (\mu^2\text{m}) = \text{Hct \% / RBC in million / mm}^3 \times 10$$

$$\text{MCH (pg)} = \text{Hb \% / RBC in million / mm}^3 \times 10$$

$$\text{MCHC (g/100mL)} = \text{Hb \% / Hct \%} \times 100$$

Serum biochemistry of *O. niloticus*

Serum total protein was estimated using the total protein kit (Biuret method) and albumin was estimated using the albumin kit (BCG dye-binding method) of Merck Specialities Pvt Ltd., Mumbai. Globulin was calculated by subtracting the albumin values from total protein. Globulin (g%) = total protein (g%) − albumin (g%).

The albumin globulin (A/G) ratio was calculated by dividing albumin values by globulin values.

Statistical analysis

Data on growth parameters are represented as mean with standard error of the mean. One-way analysis of variance (ANOVA) test using Duncan's multiple range test (Duncan

1955) was done to compare the means at 95% confidence level ($P \leq 0.05$) for growth parameters and proximate body composition of the fish. Statistical package SPSS 16 version was used and a P-value ≤ 0.05 was treated as statistically significant to reject the null hypothesis.

Results

Proximate analysis of the feeds

The crude protein content of the BSFPM feed was 29.97% with crude lipid content of 12.9%, moisture 4.47%, ash content 6.54%, and total carbohydrate as 50%. The chitin content was found to be 11.87% in BSF prepupae. The amino acid profile of the BSFPM-based feed (Table 2) showed that the 10 essential amino acids, methionine, arginine, threonine, tryptophan, histidine, isoleucine, lysine, leucine, valine, and phenyalanine are not limiting in the BSFPM-incorporated feed. The fatty acid profile of dried black soldier fly prepupae is depicted in Table 3. The total saturated fatty acid content in black soldier fly prepupae meal was 52.53%, wherein margaric acid and myristic acid dominated the fraction. The monounsaturated fatty acid dominated by oleic acid was 41.31%. The polyunsaturated fatty acids comprised only of linoleic acid which contributed to 6.16% of the total fatty acids.

Table 2 Amino acid composition of dried BSF prepupae and BSF diet expressed as % of total area

Amino acid	Dried BSF prepupae	BSFPM-based diet
Essential		
Arg*	8.09	9.78
Val	10.21	8.19
His*	8.94	10.11
Ile	9.92	8.63
Leu	14.80	14.91
Lys	-	0.24
Met	1.85	1.38
Phe	12.30	15.44
Thr	6.10	4.35
Non-essential		
Glu	2.50	3.13
Gly	9.06	8.21
Pro	2.51	2.08
Tyr	5.72	4.04
Ala	0.62	0.21
Asp	2.27	4.11
Ser	5.10	5.20

*Semi-essential amino acids

Table 3 Fatty acid composition of black soldier fly prepupae expressed as % of total area

Fatty acids	% of total area
Saturated fatty acids (SFA)	
C10:0	1.74
C14:0	18.04
C15:0	1.36
C16:0	0.48
C17:0	26.08
C18:0	4.83
∑SFA	52.53
Monounsaturated fatty acids (MUFA)	
C16:1	9.27
C18:1	32.04
∑MUFA	41.31
Polyunsaturated fatty acids (PUFA)	
C18:2	6.16
∑PUFA	6.16

Microbial and heavy metal analysis in feed

Microbial analysis of BSF prepupae showed the absence of *E. coli* and *Salmonella*. In BSFPM feed the heavy metals such as arsenic, cadmium, chromium, lead, nickel, and mercury are well within the safe limits as represented in Table 4.

Feed acceptability, survival, growth rate, and feed efficiency

The fish readily accepted all the feeds and hence no palatability issues were recorded during the study. The survival rate, feed intake, feed efficiency, and growth performances were found to be satisfactory in all the treatments (Table 5). Fish fed with control feed and BSFPM feed showed significantly higher ($P \leq 0.05$) final weight and mean weight gain when compared to other treatments. Percentage weight gain recorded significantly highest value ($P \leq 0.05$) in fish fed with control feed ($725^{c}.51 \pm 3.698$) and BSFPM feed ($706^{c}.69 \pm 8.814$) compared with other treatments. SGR also showed a similar pattern as that of PWG, with the highest values ($P \leq 0.05$) in fish fed with control feed ($2.42^{c} \pm 0.007$) and BSFPM feed ($2.39^{c} \pm 0.009$). The FCR of all the treatments differed significantly ($P \leq 0.05$) with the lowest FCR ($1.54^{a} \pm 0.081$) recorded for fish fed with control feed. Likewise FER and PER also differed significantly for treatments with significantly higher FER ($0.65^{d} \pm 0.034$) and PER ($2.11^{a} \pm 0.063$) in fish fed with control feed. The survival rate of fish ranged from 74% (T1) to 86% (T2).

There was no significant difference ($P \geq 0.05$) in crude lipid, ash, and total carbohydrate content in fish of different treatments after the feeding trial. But the crude protein content

Table 4 Heavy metal concentration in BSFPM-formulated feed

Heavy metals analysed	Heavy metal concentration in fish feed (ppm)	EU limits for heavy metals in fish feed (ppm)
Arsenic	0.6	1
Cadmium	0.25	0.5
Chromium	3.8	5
Lead	0.5	5
Nickel	0.0	NA*
Mercury	0.06	0.1

*EU limits for nickel in fish feed is not available

(57.86 ± 0.023) of fish fed with dried BSF prepupae was significantly lower ($P \leq 0.05$) than other treatments (Table 6).

The haematological and some of the serum biochemical parameters of *T. niloticus* are presented in Table 7, which shows that there is no significant difference in the Hb content, HCT value, RBC count, MCH, MCHC, and total WBC count. Hb range from 4.67 ± 0.26 to 4.97 ± 0.78 gm/dL, HCT from 23.03 ± 1.00 to 29.8 ± 1.04%, RBC count from 2.01 ± 0.05 to 2.44 ± 0.19 million/mL, MCH from 20.77 ± 0.64 to 24.87 ± 1.88 pg, and MCHC from 19.47 ± 0.44 to 20.83 ± 0.23 g/100 mL. The total WBC count range from 25,167 ± 593 to 26,367 ± 982 cells/mL. The MCV of T1 (128.5 ± 3.2) is significantly higher than the T3 (111.8 ± 6.9). The effect of different feeds on serum biochemical parameters such as serum total protein, albumin, and A/G ratio was non-significant. While the serum globulin of T1 and T3 were significantly higher than T2 and T4.

Discussion

BSF larva is reported to be a rich source of protein with a well-balanced profile particularly the essential amino acids (Makkar et al. 2014; Spranghers et al. 2017; Wang and Shelomi 2017). However, the proximate composition of BSF larvae varies widely based on the substratum on which they are reared. In our study, the protein content of BSF larvae was found to be 32.53% on dry matter basis which differed from those of earlier reports, ranging from 38.5% when reared on fruits and vegetables to 62.7% when reared on the liver as the culture medium (Nguyen et al. 2015). Caligiani et al. (2018) recorded 32% protein, 37% lipid, and 19% minerals in BSF larvae which are in conjunction with our study. The essential amino acids (EAA) are not lacking in the BSFPM and the formulated feed. Henry et al. (2015) and Liland et al. (2017) have reported that the BSF larvae have an amino acid (AA) profile equivalent to the FM except for methionine and lysine. However, in our study methionine was not lacking and the EAA composition of the pellet feed appears to be adequate with no limiting AA for Nile tilapia as observed in other freshwater fish as reported by Peres and Oliva-Teles (2007) and Magalhaes (2017).

The crude lipid content of 22.1% is well within the range of 6.63% when reared BSFL on restaurant waste (Zheng et al. 2012) and 39.2% on fruit and vegetable wastes (Nguyen et al. 2015). The published reports on proximate composition show large differences within species which is attributed to the substrates on which the insect larvae are reared (Sanchez-Muros et al. 2015).

The BSFL exoskeleton encompasses chitin which is a cell wall component in insects. This can affect the digestibility of the feed and thereby affect the growth of the fish. The chitin content of 11% in the present study was found to be within the acceptable levels in fish feed formulations as reported by Fontes et al. (2019). Tilapia fed insect meal–based feed containing 12% chitin exhibited satisfactory growth (Fontes et al. 2019). The chitin content in BSF prepupae varied from 6 to 7% (Spranghers et al. 2017) to around 9% (Caligiani et al. 2018) on dry matter basis.

Since the BSF is grown on organic wastes, there are chances of microbial contaminations. Hence it is a

Table 5 Growth parameters and feed utilisation indices of tilapia fed with experimental feeds for culture period of 90 days (mean ± standard error)

Growth parameters	Treatments			
	T1	T2	T3	C
Initial weight (g)	$4.05.^{a} \pm 0.058$	$3.96.^{a} \pm 0.035$	$3.95.^{a} \pm 0.032$	$3.96.^{a} \pm 0.015$
Final weight (g)	$29.35.^{a} \pm 0.587$	$30.28.^{a} \pm 0.315$	$31.97.^{b} \pm 0.604$	$32.69.^{b} \pm 0.158$
Mean weight gain (g)	$25.3.^{a} \pm 0.537$	$26.32.^{a} \pm 0.285$	$27.92.^{b} \pm 0.572$	$28.73.^{b} \pm 0.151$
Percentage weight gain	$624.^{a}7 \pm 7.177$	$664.^{b}64 \pm 3.918$	$706.^{c}69 \pm 8.814$	$725.^{c}51 \pm 3.698$
Specific growth rate	$2.23.^{a} \pm 0.018$	$2.28.^{b} \pm 0.009$	$2.39.^{c} \pm 0.009$	$2.42.^{c} \pm 0.007$
Food conversion ratio	$2.7.^{d} \pm 0.058$	$1.99.^{c} \pm 0.035$	$1.79.^{b} \pm 0.046$	$1.54.^{a} \pm 0.081$
Feed efficiency ratio	$0.37.^{a} \pm 0.008$	$0.50.^{b} \pm 0.009$	$0.58.^{c} \pm 0.013$	$0.65.^{d} \pm 0.034$
Protein efficiency ratio	$1.24.^{d} \pm 0.026$	$1.68.^{c} \pm 0.029$	$1.89.^{b} \pm 0.037$	$2.11.^{a} \pm 0.063$
Survival rate	$74.7.^{a} \pm 2.667$	$86.^{b} \pm 3.055$	$76.^{a} \pm 2.082$	$81.7.^{ab} \pm 2.028$

The mean values of each parameter in the same row bearing different superscripts vary significantly at 95% confidence level

Table 6 Proximate composition (on dry matter basis) of BSFPM feed and whole fish tilapia after 90-day feeding trial

Proximate composition	Treatments			
	T1	T2	T3	C
Moisture (%)	78.89.[a]±0.68	77.89.[a]±0.45	77.28.[a]±0.32	78.36[a]±0.7
Crude protein (%)	57.86.[a]±0.023	58.01.[b]±0.01	57.98.[b]±0.01	58.05.[b]±0.04
Crude lipid (%)	22.84.[a]±0.11	22.56.[a]±0.14	23.08.[a]±0.14	22.59.[a]±0.18
Ash (%)	13.97.[a]±0.11	14.01.[a]±0.15	13.91.[a]±0.15	13.73.[a]±0.06
Carbohydrate (%)	5.33.[a]±0.06	5.42.[a]±0.15	5.03.[a]±30.62	5.63.[a]±0.06

The mean values (mean ± standard error) in the same row with different superscripts vary significantly at 95% confidence level

prerequisite to conduct the microbial analysis of the insect meal to be used as ingredients in feed formulations. The study conducted by Wynants et al. (2019) reported the presence of *Salmonella* and *Bacillus cereus* in BSF larvae pointing to the importance of decontamination of the larvae before use in feed formulations. In the present investigation, the microbial analysis of dried BSF prepupae as well as BSFPM-based feed showed the absence of *E. coli* and *Salmonella*, making it safe as a feed ingredient. This could be attributed to the resistance of BSFL to ecological parameters and the ability to lessen or destroy microbes (Choi et al. 2012; Jeon et al. 2011) which makes them suitable as a feed ingredient.

Insects are susceptible to toxin accumulation or heavy metal accretion through the feed or water they consume (Marone 2016). Purschke et al. (2017) reported that BSF larvae accumulated heavy metals from the contaminated feed at lower concentrations than the initial substrate concentration, other than for cadmium and lead. This report was later backed by the findings of Shumo et al. (2019) who reported low concentrations of cadmium and lead in BSF larvae and recommended the examination of BSF larvae-incorporated feeds for possible contamination to ensure food safety. The heavy metal analysis of BSFPM-incorporated feed in the present study showed that arsenic, cadmium, chromium, lead, nickel, and mercury are well within the safe limits approved by the EU for heavy metals in fish feed.

The growth performance of the fish was satisfactory with BSFPM feed and there was no disease outbreak during the feeding trials. The experimental and control feed were readily accepted by the fish. The comparative growth analysis showed that the tilapia fed solely on BSF prepupae exhibited poor growth compared to other treatments, and this may be due to the poor digestibility of the chitin content in BSF prepupae. The study conducted by Shakil-Rana et al. (2015) has reported a similar observation in BSFL-fed tilapia fry.

The lowest FCR recorded in fish fed with fish meal feed can be due to the increased digestibility and nutrient profile of the feed especially the fatty acid profile, the polyunsaturated fatty acid which is lacking in BSFP.

Table 7 Haematological parameters of *O. niloticus* after 90 days of feeding trial

Haematological Parameters	Treatments			
	T1	T2	T3	C
Hb (gm/100 mL)	4.8.[a] ± 0.49	4.97.[a] ± 0.78	4.73.[a] ± 0.55	4.67.[a] ± 0.26
HCT (%)	28.53.[a] ± 1.04	23.03.[a] ± 1.00	26.97.[a] ± 4.64	29.8.[a] ± 1.04
RBC count (million/mL)	2.44.[a] ± 0.19	2.18.[a] ± 0.33	2.13.[a] ± 0.17	2.01.[a] ± 0.05
MCV (μ.^{2} m)	128.[a] ± 3.23	119.[ab] ± 0.85	112.[b] ± 6.97	122.[ab] ± 1.48
MCH (pg)	24.5.[a] ± 1.74	24.87.[a] ± 1.88	20.77.[a] ± 0.64	22.43.[a] ± 0.55
MCHC (g/100 mL)	19.9.[a] ± 1.04	19.47.[a] ± 0.44	19.73.[a] ± 0.47	20.83.[a] ± 0.23
Total WBC count (cells/mL)	25,767.[a] ± 841	25,800.[a] ± 1230	25,167.[a] ± 593	26,367.[a] ± 982
Serum total protein	2.86.[a] ± 0.02	2.69.[a] ± 0.08	2.78.[a] ± 0.08	2.76.[a] ± 0.02
Albumin	0.74.[a] ± 0.04	0.72.[a] ± 0.09	0.71.[a] ± 0.04	0.82.[a] ± 0.00
Globulin	2.11.[a] ± 0.05	1.94.[b] ± 0.00	2.07.[a] ± 0.04	1.96.[b] ± 0.02
A/G ratio	0.34.[a] ± 0.03	0.39.[a] ± 0.05	0.35.[a] ± 0.02	0.42.[a] ± 0.00

The mean values (mean ± standard error) in the same row with different superscripts vary significantly at 95% confidence level

Hb, haemoglobin; *HCT*, haematocrit; *RBC*, red blood cells; *MCV*, mean corpuscular volume; *MCH*, mean corpuscular haemoglobin; *MCHC*, mean corpuscular haemoglobin concentration; *WBC*, white blood cells; *A/G*, albumin/globulin

In our earlier study on Amur common carp (*Cyprinus carpio*), it was evident that the fish meal can be substituted up to 75% in formulated feed without interrupting the normal growth of the fish (Panikkar et al. 2018a). Further, Belghit et al. (2019) reported that complete substitution of fish meal with BSF larvae meal in salmon feed did not hamper the feed utilisation and growth performance of sea-water Atlantic salmon. Other studies also showed promising results when tried the BSFL incorporation in feeds for the culture of *Psetta maxima* (Kroeckel et al. 2012), *Salmo salar* (Lock et al. 2015), *Dicentrarchus labrax* (Magalhaes et al. 2017), *Danio rerio* (Vargas et al. 2018), and *Sparus aurata* (Karapanagiotidis et al. 2014).

The proximate composition analysis revealed that the crude protein content of fish fed only dried BSF prepupae was lower than other treatments. The body protein content in African catfish was not affected when replaced the fish meal with feather meal, maggot meal, and chicken offal meal but with significantly higher lipid (Adewolu et al. 2010). Lipid composition in BSFL is characterised by the high level of saturated fatty acids as reported by Liland et al. (2017) and St-Hilaire et al. (2007). However, there was no difference in crude lipid content of the fish on the completion of the feeding trials of the present study. Belghit et al. (2019) also reported similar findings in *Salmo salar* with no effect on whole body protein, lipid, as well as amino acid composition when fed with BSFL-incorporated feed replacing fish meal. The ash and carbohydrate composition also had no significant difference when compared with other treatments. Goda et al. (2007) reported no significant differences in whole-body proximate composition (gross energy and ash content) of African catfish on replacement of the fish meal in feed with other protein sources, which shows that the protein source of feed has not much influence on the protein deposition in the animal. The level of protein and lipid in BSFL can vary depending on the rearing medium as reported by Tschimer and Simon (2015), while the AA profile is not too dependent on the substrate (Spranghers et al. 2017).

Haematology and blood biochemistry are used as indicators of the health of an organism (Panikkar et al. 2018b; Manna et al. 2021). Among other extrinsic factors, diet can also affect the haematological indices as reported by Rao et al. (2015). In the present study, there is no significant difference in Hb content, HCT value, MCH, MCHC, RBC, and total WBC counts of the experimental fish which shows that the different diets have not caused any nutrition-related stress. As reported by Javed et al. (2016), a high MCV value in *Channa punctatus* is attributable to the macrocytic condition due to heavy metal exposure. In our study, the MCV values of the fish fed the BSFPM diet are non-significant with the control diet indicating the absence of any macrocytic condition. Further, the Hb, HCT, RBC count, MCH, MCHC values are similar to the values reported by Bittencourt et al. (2003) in *O. niloticus* grown under semi-intensive culture systems. These values are also in consistent with the TEC, Hb, and HCT values in *Barbodes carnaticus* as reported by Panikkar et al. (2018b) from a river system in India. The increase in WBC of fish indicates an alteration in defence mechanism as a protective response against stress (Das 1998). However, the WBC counts of BSFPM diet and control diet were comparable indicating the absence of stress related to malnutrition or other chance variables. The higher serum globulin of fish fed exclusively on BSF prepupae and the mixed diet of BSF prepupae and BSFPM feed may be associated with a stronger innate immune response in fish (Wiegertjes et al. 1996).

Conclusions

Observations from the present investigation augment well for the utilisation of BSF prepupae meal as a cost-effective protein source in formulation of freshwater fish feeds. At present, utilisation of BSFL as fish feed is being tried globally on experimental scale. Research needs to be focussed on effective utilisation of this detritivorous insect which can replace the feed ingredients in limited supply. This would enable environment friendly and sustainable way of waste reduction as well as reducing the feed cost in aquaculture industry. Inclusion of cost-effective, eco-friendly, and locally available feed ingredients in freshwater fish feed formulations could support the sustainable intensification of aquaculture practices contributing to the nutritional security.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis: Preetha Panikkar, Jesna Parakkandi, and Amala Udayakumar. Writing—original draft preparation: Preetha Panikkar and Jesna Parakkandi. Formal analysis and investigation: Feroz Khan, Mahesh Yandigeri, and Vijaykumar Muttanahalli Eregowda. Writing—review and editing and supervision: Basanta Kumar Das. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data can be made available to researchers on genuine request to improve science.

Declarations

Ethics approval The work was approved by the Institute ethical committee of ICAR-Central Inland Fisheries Research Institute, Barrackpore, India.

Consent to participate Not applicable.

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