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Impact of the Dietary *Hermetia illucens* Larvae Inclusion as a Sustainable Fish Meal Replacer on Growth Performance, Physiological Status, and Immunological Response of the Nile Tilapia

Salem Almarri, Ahmed AlSaqufi, Abdallah Mansour, Hesham Hassanien*

Animal Production and Aquaculture Department, College of Agricultural and Food Science, King Faisal University, P.O. Box 420 Hofuf 31982 Al-Hasa, Saudi Arabia

***Corresponding Author: helsanwey@kfu.edu.sa**

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ABSTRACT

The consideration of sustainable substitutes for fish meal (FM) in aquafeeds has accelerated with the global rise in demand for protein-rich food from the aquaculture sector. This study examined the impact of FM replacement at incremented levels with black soldier fly (*Hermetia illucens*) larvae meal (BSFLM) on the Nile tilapia's growth performance, physiological status, and immune response. Six isonitrogenous diets were composed of FM, which was substituted with BSFLM at 0, 20, 40, 60, 80, and 100% replacement rates and fed to juvenile tilapia (4 replicate aquaria of 20 fish per treatment group) over eight weeks. No discernible differences were seen across treatments in feed conversion ratio and other growth metrics, including specific growth rate and final body weight. However, physiological indices, including serum aspartate aminotransaminase (AST), alkaline phosphatase (ALT), creatinine, and oxidative stress markers (malondialdehyde MDA, myeloperoxidase MPO) were significantly elevated at 80–100% inclusion, indicating potential hepatic and renal stress. Conversely, immune responses such as phagocytic, lysozyme, and leukocyte proliferation were enhanced at moderate replacement levels (40–60%) but declined sharply at 100% inclusion. The findings imply that BSFLM may improve immune responses at modest inclusion levels and replace FM in the Nile tilapia diets by up to 60%, without affecting growth or health. Immune and physiological processes may be adversely affected by high inclusion rates. These results support the sustainable use of insect meals at optimal inclusion levels in aquafeeds.

INTRODUCTION

The rapid expansion of global aquaculture, driven by increasing demand for protein-rich food, has intensified the need for sustainable and affordable feed ingredients. Fish meal (FM), which has long been considered the gold standard protein source in aquafeeds because of its high digestibility and balanced amino acid profile, is becoming less viable due to its limited availability, ecological concerns related to overfishing, and volatile market prices **(Makkar *et al.*, 2014; Ozyurt *et al.*, 2018)**. This has prompted

researchers and industry stakeholders to seek alternative protein sources that may satisfy cultured fish's nutritional demands and reduce their environmental impact.

Insect-based meals, particularly those derived from black soldier fly (*Hermetia illucens*), have garnered significant attention as prospective substitutes for fish meal **(Ling *et al.*, 2025)**. Black soldier fly larval meal (BSFLM) is rich in advantageous fats, essential amino acids, high-quality protein, and several bioactive compounds, including chitin and antimicrobial peptides **(Henry *et al.*, 2015; van Huis, 2020)**. Furthermore, the larvae may be raised in large quantities on organic waste, providing a sustainable solution in line with resource-efficient feed production systems and circular economy concepts **(Siddiqui *et al.*, 2022)**.

Several researchers demonstrated the potential of BSFLM to substitute FM in aquaculture diets without compromising growth aspects or feed efficiency **(Zhou *et al.*, 2018; Tippayadara *et al.*, 2021; Sudha *et al.*, 2022; Kariuki *et al.*, 2024)**. For instance, **Belghit *et al.*, (2019)** reported that BSFLM inclusion in Atlantic salmon diets supported nutrient utilization and normal growth. Similarly, **Józefiak *et al.* (2016)** and **Devic *et al.* (2018)** observed favorable growth and feed conversion rates in the Nile tilapia and other freshwater species when BSFLM was used as a primary dietary protein source. Despite these encouraging outcomes, concerns remain regarding the physiological and immunological impacts of high BSFLM inclusion levels **(Tippayadara *et al.*, 2021)**. While moderate inclusion has been associated with enhanced immune responses and gut health, excessive dietary BSFLM may introduce challenges due to indigestible components such as chitin, high saturated fat content, or imbalanced nutrient profiles, which could impair liver function, induce oxidative stress, or suppress immunity **(Rawski *et al.*, 2020; Wang *et al.*, 2024)**. The Nile tilapia (*Oreochromis niloticus*), a globally farmed species valued for its adaptability, fast growth, and economic significance, provides an ideal model for evaluating the nutritional safety and immunological consequences of insect-based feed ingredients **(Nobrega *et al.*, 2020)**.

While previous studies have demonstrated the feasibility of BSFLM as a fish meal substitute in tilapia diets, many have primarily focused on growth performance and basic physiological parameters. The novelty of the present study lies in its comprehensive, simultaneous evaluation of growth, a detailed panel of systemic physiological status indicators (including hepatic, renal, and oxidative stress markers), and multiple facets of the innate immune response across a full spectrum (0–100%) of FM replacement. This integrated approach allows for the identification of a precise, optimal inclusion level that not only supports growth but also promotes physiological well-being and enhances immune competence. Consequently, this work provides a more holistic and nuanced understanding of the implications of BSFLM inclusion, crucial for developing sustainable aquafeeds without compromising fish health. Thus, the current study aimed to evaluate the impacts of increased substitution rates of FM with BSFLM on growth indices, physiological traits, and immune response parameters in the Nile tilapia. The findings

declare the sustainable development of insect-based aquafeeds while maintaining fish health and production efficiency.

MATERIALS AND METHODS

Ethical declaration

The experimental study protocols were accredited by the ethical research council of King Faisal University in Saudi Arabia (Accreditation number: KFU-REC-2023-APRIL-ETHICS95).

BSFLM and experimental diets

A dried BSFLM for animal feed purposes was obtained from a commercial supplier (Hindusthan Protein, Tiruppur, Tamil Nadu, India). The BSFLM was ground to fine particles using an electric miller and the outcome was kept at 4°C for later use. The nutritional value of the BSFLM per 100g of dry matter, according to the pamphlet of the commercial supplier, was 40g protein, 30g fat, 4.5g fiber, 3.6g calcium, 0.9g phosphorus, and 2.3 MJ gross energy. The experimental diets were prepared by substituting BSFLM for FM at a rate of 0 (control), 20, 40, 60, 80, and 100%, respectively. The BSFL powder was mixed well with the components of the diet to reach the final BSFL concentration in the experimental diets, and then properly compacted at a temperature of 100°C into 2-mm-diameter pellets through an extruder machine. Following a 24-hour drying period at room temperature, the diet pellets were kept in plastic bags at 4°C. Ingredients and nutritional assays of the experimental diets were determined by AOAC techniques **(AOAC, 2005)** , as displayed in Table (1).

Experimental protocol

Four hundred eighty fingerlings of the Nile tilapia (*Oreochromis niloticus*) were acquired from an aquaculture company in Riyadh, Saudi Arabia (Saqua, Industrial City II). The fish were first maintained for two weeks in a glass aquarium of $1m^3$ capacity for acclimation on the control diet and basic Nile tilapia environmental conditions, which included a 12L/12D photoperiod cycle, 6.0mg/ L DO, 7.5 pH, and 28°C **(Tippayadara *et al.*, 2021)**. After that, fish fingerlings (22.31±0.156 g average weight) were captured and transferred into 24 aquaria (20 fish per aquarium of 100L volume) supplied with the same environmental conditions and aerated by an air compressor. Fish in each four aquariums were allocated as replicates to one of the six treatment groups according to FM replacement with BSFLM in the experimental diets at a rate of 0, 20, 40, 60, 80, and 100%, respectively. Fish were fed two meals of the experimental diets, quantified every week as 3% of the aquarium fish biomass, and introduced at 8:00 and 15:00 h daily for eight consecutive weeks. Throughout the experiment, 75% of the aquarium's water was siphoned out daily to eliminate waste and leftover feed to preserve the water's quality. This was immediately replaced with fresh, adequately aerated water. As detailed below,

growth performance traits, physiological indices, and immunological response were evaluated for all treatment groups.

Table 1. Nutritional analysis of experimental diets introduced to the Nile tilapia (*Oreochromis niloticus*) fingerlings

Ingredient (g/kg as fed)	FM replacement rate with BSFLM					
	0%	**20%**	**40%**	**60%**	**80%**	**100%**
BSFL meal	0	36	72	108	144	180
Fish meal	180	144	108	72	36	0
Soybean meal	320	310	300	290	285	280
Yellow corn	315	315	315	315	315	315
Wheat bran	60	60	60	60	60	60
Corn gluten	52	62	72	82	87	92
Vegetable oil	50	50	50	50	50	50
Calcium mono-hydrogen phosphate	5	5	5	5	5	5
Lysin	2	2	2	2	2	2
Methionine	2	2	2	2	2	2
Premix [1]	14	14	14	14	14	14
Nutritional analysis (per kg DM)						
Dry matter	908.0	906.5	902.8	907.0	907.5	904.6
Crude protein	349.8	345.5	347.2	347.0	339.8	338.2
Crude lipids	68.3	68.4	67.5	67.3	68.0	67.6
Total ash	75.0	75.2	73.8	72.5	70.7	69.8
Crude fiber	30.7	32.2	29.6	30.1	27.8	28.1
Calcium	8.9	8.7	7.8	7.1	6.8	6.0
Available phosphorus	9.1	9.0	8.5	9.0	8.1	7.9

[1] Contents per kg: 400mg α-tocopherol acetate, 4000 IU cholecalciferol, 40000 IU retinol, 80µg cyanocobalamin, 40mg riboflavin, 30mg thiamine, 12mg menadione, 30mg pyridoxine, 10mg folic acid, 500 mg ascorbic acid, 3mg biotin, 500mg inositol, 100mg pantothenic acid, 250mg ferric citrate, 60mg zinc carbonate, 40mg potassium sulfate, 40mg manganese sulfate, 12 mg Copper sulfate, 10 mg magnesium oxide, 0.24mg sodium selenite, 0.4mg potassium iodide, and 0.2mg cobalt.

Growth performance traits

Feed intake (FI) was determined by collecting and drying the residual feed, then subtracting this quantity from the total feed provided. The mean body weight (BW) of the fish in each tank was evaluated at the commencement and conclusion of the experiment (8 weeks) to ascertain the initial (IBW) and final (FBW) body weight, and compute the body weight gain (BWG) as the difference between FBW and IBW. The additional growth performance metrics for each replicate within the treatment groups were then calculated using the following formulas: Feed conversion ratio (FCR) = FI / BWG; specific growth rate (SGR) = 100 × (Ln FBW – Ln IBW) / d, where d represents the duration of the feeding cycle in days and Ln denotes the natural logarithm of the values. To assess the cumulative mortality rate (CMR%), the total number of deceased fish

throughout the feeding experiment was calculated as a percentage of the initial number of fish in each tank replication.

Physiological status indices

Per the trial procedure outlined by **Martins *et al.*, (2016)**, three fish were randomly captured from each replication of the treatment group during the 8-week feeding experiment and were immersed in a tank containing 100mg tricaine methane sulfonate (MS-222) per liter of distilled water. After fish anesthesia, blood was withdrawn from the caudal vein and samples were preserved slantly in tubes at 4°C overnight. After clotting, the serum was separated via 1075 × g centrifuging for 20 minutes and preserved at -20°C for further assays. Physiological biomarkers, including creatinine (CRT), alanine (ALT) and aspartate (AST) aminotransferase enzymes, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), myeloperoxidase (MPO), and malondialdehyde (MDA), were evaluated using commercially available colorimetric test kits (Elabscience Biotechnology Inc., Houston, TX, USA), following the manufacturer's guidelines. The AST and ALT activities were evaluated by generating oxaloacetic acid and pyruvic acid, respectively, which conjugate with phenyl hydrazine to generate a reddish-brown phenylhydrazone detectable at 510nm **(Desai & Desai, 2008; Campos *et al.*, 2011)**. The sarcosine oxidase method was used to quantify CRT, yielding a pink chromophore observable at 515nm **(Nishida *et al.*, 2015)**. The assessment of ALP activity was conducted with the methodology outlined by **Fernandez and Kidney (2007)**. This technique hydrolyzes benzene disodium phosphate to get phenol and phosphoric acid, which react with potassium ferricyanide and 4-aminopyrine to produce a red quinone compound detectable at 520nm. LDH activity was assessed by catalyzing the transformation of lactic acid into pyruvate, which then reacted with phenyl hydrazine to form a reddish-brown complex detectable at 540nm **(Liao *et al.*, 2021)**. The ability of MPO to reduce hydrogen peroxide and to generate a yellowish product containing o-dianisidine, visible at 460 nm, was used to quantify its levels **(Mihaila *et al.*, 2021)**. The MDA content was quantified by reacting with Thio-barbituric acid to produce a red adduct detectable at 532nm **(Arora *et al.*, 2022)**. All absorbance measurements were conducted with an automated microplate scanner (ELx808TM, BioTek Instruments, Winooski, Vermont, USA).

Immune response parameters

Total white blood cell (TWBC) count and heterophil to lymphocyte (H/L) ratio

Blood samples from three fish per replication were taken into tubes and gently mixed with 10% ethylenediaminetetraacetic acid (EDTA) as an anticoagulant after the eight-week feeding period. Following the methods outlined by **Rawling *et al.* (2009)**, a hemocytometer Bright-Line™ (American Optical, Buffalo, NY, USA) was used to count the TWBC in a small aliquot of whole blood. Leukocyte differential counts were also

performed using a different drop of blood. Hema-3 solutions (Fisher Scientific, Pittsburgh, PA, USA) were added to the blood smear for staining, and then 200 leukocytes were found and classified. Next, H and L cells were counted and the H/L ratio was computed **(Zhang *et al.*, 2009)**.

Peripheral blood leukocyte (PBL) stimulation index

Blood samples from three fish per replication were mixed with 10% EDTA. Following **Carvalho *et al.* (2018)** with minor adjustments, the samples were used for the PBL proliferation test. After extending the blood with PBS (1:2 v/v), it was centrifuged for 30 minutes at room temperature using an equivalent amount of Histopaque-1077 medium (Sigma, MA, USA) at 400 × g. The interface layer's PBLs were removed and given two sterile PBS washes using 600 × g centrifugation for ten minutes each. The cell pellet was reconstituted in 1mL of RPMI-1640 medium (Invitrogen Corp., Grand Island, NY, USA) and counted after staining with trypan blue to scan the viable cells (>95%). Triplicate 96-well U-bottom plates were used to disseminate workable cells at a final concentration of 3×10^6 cells/mL. Ten µg/mL of lipopolysaccharide (LPS, Sigma, MA, USA) was introduced to stimulate cells, whereas control wells received just RPMI-1640 media. After 18 hours of incubation at 27°C, plates were treated with MTT for 4 hours. Following incubation, plates were centrifuged for five minutes at 110 × g; the supernatant was removed, and 100µL of DMSO was used to dissolve the generated formazan crystals. The PBL stimulation index (PBL-SI) was ultimately evaluated by measuring the absorbance at 570nm for both stimulated and non-stimulated control cells using a microplate automated scanner (Bio-Rad 550, Laboratories Inc., USA).

Phagocytosis test

Employing a slightly altered methodology from **Almarri *et al.* (2023)**, blood samples were collected from three fish per replication within the treatment groups after the 8-week feeding trial. Leukocytes were isolated and incubated with TRITC-labeled *Candida albicans* suspension (Sigma, MA, USA) at a ratio of 1:4. The mixes were incubated at 37°C for 30 minutes in 24-well plates covered with gelatin plasma. Total phagocytes and those that engulfed at least one yeast cell during incubation were enumerated using a hemocytometer under an inverted microscope. Phagocytic Activity (PA %) = (number of phagocytes engulfing yeast / total phagocytes %).

Lysozyme test

Employing the turbidimetric method established by **Bae *et al.* (2012)**, lysozyme activity (LA) was assessed based on its ability to induce cell wall lysis of Gram-positive bacteria. One suspension milliliter of *Micrococcus lysodeikticus* (Sigma-Aldrich, Burlington, MA, USA) was combined with 50 microliters of serum (three samples per

replicate). During a 30-minute period, absorbance at 450nm was recorded using a spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK) at intervals of 0.5 and 4.5 minutes. The absorbance decreased by 0.001 per unit of lysozyme activity per minute.

Statistical analysis

Fish served as the number of observations for physiological and immunological parameters (n= 12 fish per treatment). In contrast, tanks were designated as the experimental units for growth data (n= 4 observations per treatment). All datasets underwent the Shapiro-Wilk test for normality before statistical analysis. A one-way analysis of variance (ANOVA) accompanied by Tukey's post hoc test was performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA, 2013) to evaluate disparities across treatment groups. Furthermore, polynomial contrast analysis was used to assess linear and quadratic trends associated with the increasing quantities of BSFLM replacing FM in the diet. A P-value less than 0.05 was considered statistically significant.

RESULTS

Growth performance

Table (2) shows how the growth indices of the Nile tilapia is affected when FM is substituted with BSFLM. There were no discernible changes among treatment groups in the FBW, BWG, SGR, FI, FCR, or CMR (P> 0.05). Additionally, polynomial comparison analysis revealed no significant linear or quadratic trends in the growth performance metrics (P> 0.05).

Table 2. Impact of replacing black soldier fly meal for fish meal at various levels on the growth indices of the Nile tilapia

Replacement rate	IBW (g)	FBW (g)	BWG (g)	SGR (%)	FI (g)	FCR	CMR (%)
0%	22.24	45.14	22.90	1.26	49.10	2.14	0.00
20%	22.29	45.75	23.46	1.28	48.12	2.05	1.25
40%	22.26	45.78	23.52	1.29	48.46	2.06	0.00
60%	22.38	46.18	23.80	1.29	47.98	2.02	0.00
80%	22.26	46.47	24.21	1.31	49.28	2.04	1.25
100%	22.18	46.33	24.15	1.32	47.54	1.97	2.50
SEM	1.156	2.072	2.350	0.136	2.296	0.109	1.318
***P*-value**							
Overall	0.997	0.637	0.343	0.836	0.755	0.988	0.349
Linear	0.501	0.532	0442	0.735	0.904	0.419	0.126
Quadratic	0.504	0.193	0.112	0.334	0.488	0.267	0.257

Data represent the means ± standard error of means (SEM) of 4 replicate aquaria per treatment group. Superscript letters rank the differences between the means within the same column at an overall p-value < 0.05. Linear and quadratic effects for increasing the replacement rate of FM with BSFLM in the diet on each parameter are significantly presented at P<0.05. Abbreviations: BSFLM, black soldier fly larvae meal;

BWG, body weight gain; CMR, cumulative mortality rate; FBW, final body weight; FCR, feed conversion ratio; FI, feed intake; FM, fish meal; IBW, initial body weight; SGR, specific growth rate.

Physiological indicators

As seen in Table (3), the dietary FM replacement with BSFLM had a linear and/or quadratically significant impact ($P< 0.05$) on several physiological parameters. Compared to the control, both ALT and AST activities significantly increased in the 80 and 100% replacement groups ($P< 0.001$). The 100% BSFLM group had the highest AST (69.67 IU/L) and ALT (29.71 IU/L) values, while the 60% or lower replacement levels had the lowest values. Significant variations were also seen in serum creatinine (CRE) levels, with the 60% group having the lowest value (49.62 µmol/L) and the 100% BSFLM group having the highest value (60.56 µmol/L) ($P< 0.001$). LDH was the greatest in the 100% group and the lowest at the 60% level ($P= 0.007$), and ALP levels were markedly higher in the 100 and 80% replacement groups ($P< 0.001$). Considerable ($P< 0.001$) effects were seen in the redox-related enzymes MPO and MDA, which peaked in the 100% BSFLM group (86.60 U/L and 5.61 µmol/L, respectively) and decreased in the 60% group (61.77 U/L and 3.04 µmol/L, respectively) (Fig. 1).

Table 3. Impact of replacing black soldier fly meal for fish meal on the physiological status of the Nile tilapia

Replacement rate	AST, IU/L	ALT, IU/L	CRE, µmol/L	ALP, U/L	LDH, U/L	MPO (U/L)	MDA (µmol/L)
0%	54.35 ^{c}	22.32 ^{c}	54.93 ^{b}	46.15 ^{abc}	154.14 ^{ab}	74.48 ^{c}	4.20 ^{b}
20%	54.25 ^{c}	22.36 ^{c}	54.82 ^{b}	45.15 ^{bc}	149.97 ^{ab}	74.57 ^{c}	4.20 ^{b}
40%	51.57 ^{cd}	20.81 ^{c}	51.28 ^{bc}	44.08 ^{c}	148.30 ^{ab}	63.52 ^{d}	4.24 ^{b}
60%	50.35 ^{d}	19.34 ^{c}	49.62 ^{c}	44.41 ^{c}	146.53 ^{b}	61.77 ^{d}	3.04 ^{c}
80%	64.23 ^{b}	26.10 ^{b}	49.94 ^{c}	48.71 ^{ab}	161.38 ^{a}	82.67 ^{b}	5.21 ^{a}
100%	69.67 ^{a}	29.71 ^{a}	60.56 ^{a}	48.85 ^{a}	162.16 ^{a}	86.60 ^{a}	5.61 ^{a}
SEM	1.294	1.190	1.508	1.244	5.058	1.199	0.220
***P*-value**							
Overall	<0.001	<0.001	<0.001	<0.001	0.007	<0.001	<0.001
Linear	<0.001	<0.001	0.189	0.001	0.018	<0.001	<0.001
Quadratic	<0.001	<0.001	<0.001	0.001	0.007	<0.001	<0.001

Data represent the means ± standard error of means (SEM) of 12 replicate samples per treatment group. Superscript letters rank the differences between the means within the same column at an overall *P*-value <0.05. Linear and quadratic effects for increasing the replacement rate of FM with BSFLM in the diet on each parameter are significantly presented at $P<0.05$. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransaminase; BSFLM, black soldier fly larvae meal; CRE, creatinine; FM, fish meal; LDH, lactate dehydrogenase; MDA, malondialdehyde; MPO, myeloperoxidase.

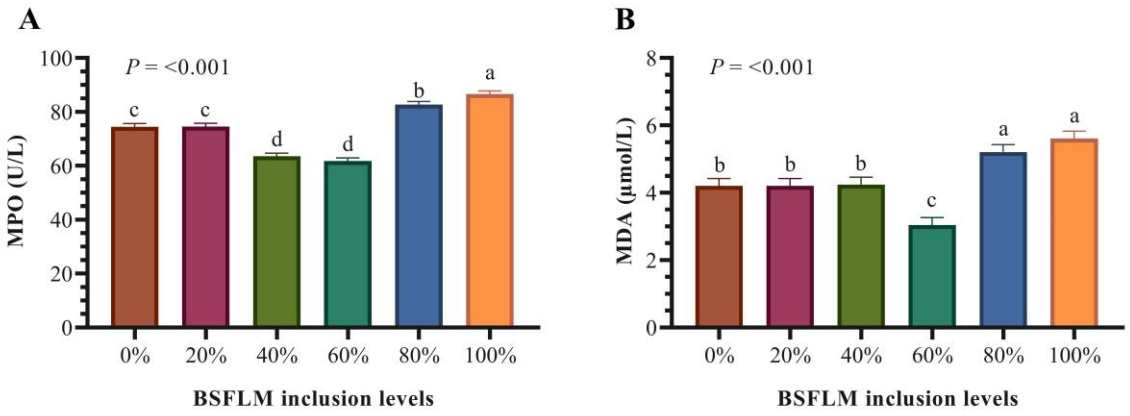

Fig. 1. Effect of replacing fishmeal by increasing levels of black soldier fly larvae meal (BSFLM) on myeloperoxidase (MPO; A) and malondialdehyde (MDA; B) of the Nile tilapia, *Oreochromis niloticus*

Immune response

Table (4) shows how the immune responses of the Nile tilapia are affected when BSFLM is added to their meals instead of FM. For every immune response indicator, linear and quadratic trends were significant ($P< 0.05$). Compared to the control fish, the TWBC count climbed linearly ($P< 0.05$) to the maximum values at 60% replacement rate and subsequently quadratically fell to the lowest value at 100% replacement rate.

Table 4. Impact of replacing black soldier fly meal for fish meal on the immune response of the Nile tilapia

Replacement rate	TWBC (10^3/µL)	H/L ratio	PBL-SI	PA (%)	LA (U/mL)
0%	4.46 ^{bc}	0.44 ^{b}	3.11 ^{b}	11.78 ^{b}	15.37 ^{bc}
20%	4.88 ^{b}	0.45 ^{b}	2.12 ^{b}	16.13 ^{b}	18.00 ^{b}
40%	5.69 ^{a}	0.44 ^{b}	3.67 ^{a}	20.78 ^{a}	22.97 ^{a}
60%	6.23 ^{a}	0.41 ^{c}	4.14 ^{a}	26.74 ^{a}	25.37 ^{a}
80%	3.88 ^{cd}	0.52 ^{a}	3.20 ^{b}	14.81 ^{c}	13.54 ^{cd}
100%	3.50 ^{d}	0.53 ^{a}	1.13 ^{c}	7.43 ^{d}	11.71 ^{d}
SEM	0.552	0.010	0.584	0.547	2.772
***P*-value**					
Overall	0.022	<0.001	0.003	<0.001	0.006
Linear	0.014	<0.001	0.044	<0.001	0.059
Quadratic	<0.001	<0.001	<0.001	<0.001	<0.001

Data represent the means ± standard error of means (SEM) of 12 replicate samples per treatment group. Superscript letters rank the differences between the means within the same column at an overall *P*-value <0.05. Linear and quadratic effects for increasing the replacement rate of FM with BSFLM in the diet on each parameter are significantly presented at $P<0.05$. Abbreviations: BSFLM, black soldier fly larvae meal;

FM, fish meal; H/L ratio, heterophil to lymphocyte cell ratio; LA, lysozyme activity; PA, phagocytic activity; PBL-SI, peripheral blood leukocyte stimulation index; TWBC, total white blood cells.

In comparison with the control group, the addition of BSFLM in the fish meals had a linear and quadratically significant impact on the H/L ratio, with the greatest values seen in the 80 and 100% groups and the lowest values in the 60% group ($P< 0.05$). At 60% replacement, the PBL-SI peaked, and at 100% replacement, it declined ($P< 0.05$). Likewise, the 60% group had the greatest PA, whereas the 100% replacement group had the lowest ($P< 0.05$). LA showed a similar trend, peaking at 60% and dropping significantly after 100% replacement ($P< 0.05$).

DISCUSSION

Fish nutritionists are committed to researching innovative feeding techniques to maximize the production of the Nile tilapia since it is a well-liked, affordable, and nutrient-dense source of animal protein **(Dawood *et al.*, 2020; Mansour, 2025)**. Despite being a common source of protein in aquafeeds for a long time, FM is becoming less viable and unsustainable **(Ozyurt *et al.*, 2018)**. The present research assessed how the addition of BSFLM as a sustainable FM substitute in the Nile tilapia diets affected their growth performance, physiological state, and immunological response.

BSFLM is a nutritionally sufficient FM replacement in tilapia diets, as shown by the absence of significant changes in FI, FBW, BWG, SGR, and FCR across treatments. According to earlier research, BSFLM may be used in place of FM in tilapia and other fish species without affecting growth **(Devic *et al.*, 2018; Dietz & Liebert, 2018; Ushakova *et al.*, 2018; Tippayadara *et al.*, 2021)**. These results are consistent with those findings. The tilapia groups' similar feed intakes indicate that the diets are palatable, which confirms earlier findings that different aquaculture species accept black soldier fly larvae **(Dumas *et al.*, 2018; Belghit *et al.*, 2019; Abdel-Tawwab *et al.*, 2020; Fisher *et al.*, 2020; Li *et al.*, 2020)**.

Although BSFLM incorporation into tilapia diets seems applicable up to 100%, without negatively affecting growth and feed efficiency; such high inclusion levels may impair physiological and immune parameters. The elevated serum AST, ALT, and creatinine levels in the 80 and 100% replacement groups suggest potential hepatic and renal stress at high inclusion levels of BSFLM **(Sangsawang *et al.*, 2024)**. The rise in ALT and AST, particularly in the 100% BSFLM group, may indicate hepatocellular damage or altered metabolic activity due to potential accumulation of indigestible components such as chitin or saturated fats found in insect meals **(Fontes *et al.*, 2019)**. In contrast, the moderate intake of chitin in the 40 and 60% groups may initiate chitinolytic enzymes activities that improve gut health and digestion **(Rimoldi *et al.*, 2019)**. Increased levels of ALP and LDH, especially at higher replacement rates in the current study, may further reflect tissue stress and metabolic disturbances **(Chang *et al.*, 2020; Hassanien *et al.*, 2023)**. Moreover, the pronounced elevation of MPO and MDA at 80 and 100%

BSFLM inclusion indicates enhanced oxidative stress and inflammation **(Alrashada *et al.*, 2023; Hassanien *et al.*, 2023)**. This suggests that although BSFLM is a viable FM substitute, excessive inclusion may exceed the physiological adaptability of the Nile tilapia, potentially due to residual antimicrobial peptides, lipid peroxidation products, or high chitin content in insect meal **(Ling *et al.*, 2025)**.

The immune response data further highlight the dose-dependent effect of BSFLM on the Nile tilapia. Moderate inclusion levels (40–60%) significantly improved immune markers, including TWBC, phagocytic activity, lysozyme activity, and PBL stimulation index. These findings are consistent with studies showing that low-to-moderate levels of insect meal can enhance innate immune responses in fish by acting as natural immunostimulants **(Xiao *et al.*, 2018; Foysal *et al.*, 2019; Tippayadara *et al.*, 2021)**. This may be due to the likely immunostimulatory compounds in insect meals, including lauric acid, antimicrobial peptides, and chitin-derived β-glucans **(van Huis, 2020; Tippayadara *et al.*, 2021)**. It has been reported that dietary BSFLM comprises large levels of chitin implicated in boosting the number of microbial colonies in the fish gut, therefore functioning as prebiotic compounds that might produce immunomodulatory consequences on fish **(Bruni *et al.*, 2018; Terova *et al.*, 2019)**. However, immune performance sharply declined at 100% replacement with BSFLM, evidenced by reduced PBL stimulation, lysozyme activity, and phagocytic function. The increase in H/L ratio at 80 and 100% BSFLM inclusion also indicates elevated physiological stress, commonly associated with immune suppression or suboptimal dietary conditions **(Goessling *et al.*, 2025)**. This immune suppression at higher levels of BSFLM may be linked to nutrient imbalances, excessive chitin, or the presence of bioactive substances that modulate immune function negatively at high concentrations **(Rawski *et al.*, 2020)**.

CONCLUSION

This research shows that BSFLM can successfully replace up to 60% of the FM in the Nile tilapia diets, without affecting survival, feed efficiency, or growth performance. The immunomodulatory advantages of BSFLM are suggested by moderate inclusion levels (40–60%), which even improved immune function and supported physiological wellness. However, greater replacement rates (80–100%) were linked to worse immunological responses and greater stress markers, suggesting potential nutritional or metabolic abnormalities at excessive inclusion levels. Our results indicate that to improve fish performance and environmental sustainability in aquaculture systems, BSFLM could be used as a sustainable substitute for FM in aquafeeds, especially at moderate inclusion rates.

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