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Optimizing the Nile Tilapia Nutrition: Effects of Black Soldier Fly Larvae Meal on Growth, Blood Biochemistry, and Growth-Related Genes

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ABSTRACT

Insect-based feed ingredients offer a sustainable approach to enhancing aquaculture productivity. This study examined the effects of dietary black soldier fly larvae meal (BSFLM) at 0, 15, 30, and 45% on growth performance, feed efficiency, blood biochemical parameters, and growth-related gene expression in the Nile tilapia (*Oreochromis niloticus*). A total of 120 fingerlings (36.5 ± 0.68 g) were distributed into 12 aquaria (10 fish per tank) and fed the experimental diets. BSFLM contained 35.4% crude protein, 30% ether extract, 17.95% ash, and 5511 kcal/kg dry matter. Fish receiving 15–45% BSFLM showed significant improvements in final weight, daily gain, specific and relative growth rates, feed intake, protein intake, protein efficiency ratio, and feed conversion ratio. Biochemical analysis revealed elevated serum protein and globulin levels, while ALT, AST, uric acid, creatinine, and glucose were reduced in the 15% BSFLM group. Cholesterol remained stable at 15% inclusion but increased at higher levels. Gene expression analysis indicated no significant upregulation of IGF1, with the highest levels in control and 15% groups, whereas GH expression peaked at 45% BSFLM. These results suggest that BSFLM enhances growth performance, feed utilization, and metabolic health of the Nile tilapia, with 15% dietary inclusion being optimal.

INTRODUCTION

The rapid growth of the global population has intensified the demand for safe and sustainable food production, placing pressure on aquaculture systems to expand efficiently while minimizing environmental impacts **(Salin *et al.*, 2018)**. Traditionally, fishmeal has served as the primary protein source in aquafeeds due to its balanced amino acid profile and high digestibility. However, rising costs, declining availability of marine resources, and environmental concerns have prompted the search for alternative protein sources to support the continued growth of aquaculture **(Furuya *et al.*, 2010)**. This challenge is particularly pronounced for intensively farmed species, including omnivorous fish such as the Nile tilapia and carp, and carnivorous species like Atlantic salmon, rainbow trout, and European sea bass, which require protein-rich diets for optimal growth. Insects have recently emerged as promising aquafeed ingredients, offering high-quality protein with a favorable amino acid composition, helping to overcome the limitations of plant-based proteins and supporting proper growth and metabolic performance in fish **(Fitches & Smith, 2018)**. Depending on species and developmental stage, insect meals typically contain 40–75% protein, alongside essential fatty acids, vitamins, and minerals **(Henry *et al.*, 2015)**. Their lipid fraction, ranging from 10–38% depending on rearing and processing methods, further enhances nutritional and industrial value **(Barroso *et al.*, 2014)**. Among insect species, the black soldier fly (*Hermetia illucens*) has become a leading candidate for large-scale feed production. Its larvae contain approximately 40% protein and 30% lipids **(Xu *et al.*, 2014)** and can be reared on organic waste, converting low-value biomass into nutrient-rich feed while contributing to waste reduction **(Spranghers *et al.*, 2017)**. Numerous studies have shown that black soldier fly larvae meal (BSFLM) can replace fishmeal in aquafeeds for salmonids, tilapia, and shrimp without compromising growth, nutrient digestibility, or fillet quality **(Dumas *et al.*, 2018; Belghit *et al.*, 2019; Yildirim-Aksoy *et al.*, 2020; Caimi *et al.*, 2021; Weththasinghe *et al.*, 2021)**. Additionally, bioactive compounds such as chitin and chitosan in insect meals may enhance immunity and disease resistance **(Yildirim-Aksoy *et al.*, 2020; Tippayadara *et al.*, 2021)**. The Nile tilapia (*Oreochromis niloticus*) is one of the most widely farmed fish globally, prized for its fast growth, environmental adaptability, and high market demand **(El-Sayed, 2006; FAO, 2022)**. Ensuring the sustainable expansion of tilapia farming requires alternative, cost-effective, and nutritionally balanced feed sources that not only meet basic dietary requirements but also influence physiological and molecular growth mechanisms. The growth hormone (GH)/insulin-like growth factor 1 (IGF1) axis is central to growth regulation, with GH stimulating hepatic IGF1 production, which in turn promotes nutrient utilization, protein synthesis, and cell proliferation **(Wood *et al.*, 2005; Reindl & Sheridan, 2012)**. Dietary protein sources can modulate GH and IGF1 expression at transcriptional and translational levels **(Dawood & Koshio, 2020)**, and recent evidence suggests that BSFLM may enhance growth performance and regulate this axis through its balanced amino acids, functional lipids such as lauric acid, bioactive peptides, and chitin **(Katya *et al.*, 2017; Mousavi & Zahedinezhad, 2020; Ravi & Venkatesan, 2020)**. Based on this context, the present study evaluated the effects of graded dietary BSFLM levels on growth performance, feed efficiency, blood biochemical parameters, and expression of GH and IGF1 genes in the Nile tilapia fingerlings. The findings aim to provide insights into the nutritional and functional potential of BSFLM as a sustainable protein alternative in aquaculture.

MATERIALS AND METHODS

The present study investigated the effects of incorporating black soldier fly larvae meal (BSFLM) at varying dietary levels on the growth performance, blood biochemical parameters, and gene expression of the Nile tilapia fingerlings. The experiment was conducted at the Fish Experimental Laboratory, Animal Production Department, Agricultural Biological Research Institute, in collaboration with the Hydrobiology Department, Veterinary Research Institute, and the Cell Biology Department, Biotechnology Research Institute, National Research Centre, 33 El-Bohouth Street, Dokki, Cairo, Egypt (P.O. 12622).

Experimental unit

A total of 120 Nile tilapia (*Oreochromis niloticus*) fingerlings were acclimated prior to the feeding trial and randomly assigned to 12 glass aquaria (80 × 40 × 30 cm), with 10 fish per tank. The fingerlings, obtained from the Abbassa Fish Hatchery, Sharkia Governorate, Egypt, had an initial mean body weight of 365 ± 0.69 g per aquarium. Each tank was supplied with 60L of dechlorinated tap water, and fish were fed a control diet for two weeks during the acclimation period before the start of the experimental trial.

Experimental design and diets

The formulation and proximate composition of the experimental diets are shown in Table (1). Four experimental diets were prepared to evaluate the effects of BSFLM as untradional protein source on growth, blood biochemistry, and growth-related genes. A basal control diet was formulated to meet the nutritional requirements of the species, and three additional diets were produced by incorporating BSMFLM at 15, 30, and 45% of diets.

All dry ingredients (Table 1) were finely ground to pass a 500 µm sieve and thoroughly mixed in a mechanical blender for 5 min. Fish oil and approximately 30–35 % water were gradually added to obtain a homogeneous dough. For the supplemented diets, the required amount of nano-curcumin powder was first premixed with a small portion of the basal feed to ensure uniform distribution, then blended into the full batch.

Table 1. Composition of the different experimental diets

Item	**Experimental diets**			
	Zero BSFLM	**15% BSFLM**	**30% BSFLM**	**45% BSFLM**
	T_1	**T_2**	**T_3**	**T_4**
Composition of tested diets				
Black soldier fly larvae meal	**0**	**15**	**30**	**45**
Soybean meal (44%)	**40**	**30**	**9**	**3**
Protein concentration (56%)	**17**	**14**	**18**	**12**
Ground Yellow corn (8%)	**28**	**26**	**28**	**24**
Wheat bran (13%)	**10**	**10**	**10**	**11**
Vegetable oil	**3**	**3**	**3**	**3**
Vitamin and Minerals mixture*	**2**	**2**	**2**	**2**

*Vitamin and Minerals mixture: contained Vit. A (E672) (IU) 876.19, Vit. D3 (IU) 1141.39, Vit. E 114.30, Vit. K3 7.55, Vit. B1 13.71, Vit. B2 11.44, Vit. B6 15.33, Vit. B12 0.03, Niacin 60.96, Calpan 30.48, Folic Acid 3.04, Biotin 0.37, Vit. C 11.44, Selenium 0.27, Manganese 19.04, Iron 9.15, Iodine 0.77, Zinc 76.19, Copper 3.04, Cobalt 0.37, Choline Chloride 457.14, and Antioxidant 95.23 (Vit. vitamin; IU international unit).

BSFLM: black soldier fly larvae meal.

The dough was pelleted using a laboratory pelletizer fitted with a 2mm die, producing pellets approximately 2–3 mm long. Pellets were dried in a forced-air oven at 55°C until moisture content was below 10 %, then cooled to room temperature. After drying, pellets were stored in airtight, light-proof polyethylene bags at 4°C until use.

Representative samples from each diet were analyzed for proximate composition (moisture, crude protein, lipid, ash, and fiber) following **AOAC (2019)** procedures.

The Nile tilapia (*Oreochromis niloticus*) fingerlings were randomly assigned to four dietary treatments:

T1 (Control): Diet without black soldier fly larvae meal (BSFLM).

T2: Diet containing 15% BSFLM.

T3: Diet containing 30% BSFLM.

T4: Diet containing 45% BSFLM.

The feeding trial lasted for 56 days, from early May to the end of June 2024.

Parameters of growth performance (NRC, 2001)

Body weight gain (BWG) = Final weight - Initial weight.

Survival rate (SR%) = Number of fish at final / Number of fish at start x100.

Specific growth rate (SGR) =

[In final weight (g) - In initial weight (g)] / Experimental days *100

Calculation of feed conversion ratio (FCR)

FCR = total dry matter intake, (TDMI), g / total body weight gain (TBWG), g.

Calculation of crude protein efficiency ratio (CPER)

(PER) = total body weight gain (TBWG), g / total crude protein intake (TCPI), g.

Feed efficiency

Feed efficiency (FE %) = [weight gain (g) / feed intake (g)]

Analytical procedures

Analysis of tested diets and fish body composition analyzed according to **AOAC** (**2016**)

Blood sampling

Blood samples were drawn from the caudal vein of experimental fish using 3mL sterile syringes after anesthesia with clove oil (0.5mL L^{-1}). The samples were placed into clean, dry centrifuge tubes and allowed to clot at room temperature before centrifugation at 3000 rpm for 15min. The resulting serum was separated and stored at −20°C for subsequent biochemical analyses.

Biochemical assays

Serum biochemical parameters were analyzed using standard procedures. Total protein was determined according to **Cannon *et al*. (1974)**, albumin following **Tietz (1990)**, and globulin was calculated as the difference between total protein and albumin concentrations. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured as described by **Reitman and Frankel (1957)**. Total cholesterol was assessed following **Ellefson and Caraway (1976)**, and glucose according to **Caraway and Watts (1987)**. Uric acid and creatinine concentrations were determined following **Tietz (1990)**.

All assays were performed using commercial diagnostic kits (Spectrum Diagnostics, Egypt). Colorimetric measurements were carried out with an Agilent Cary UV-Vis spectrophotometer (100/300 Series) in accordance with the manufacturer’s guidelines.

Gene expression analysis

Molecular responses to dietary BSFLM were evaluated; quantitative real-time PCR (qPCR) was performed on liver tissues. At the end of the feeding trial, three fish per replicate (nine fish per treatment) were randomly selected and euthanized using an overdose of clove oil (1mL L^{-1}). Liver and head kidney tissues were excised, immediately snap-frozen in liquid nitrogen, and stored at −80°C until RNA extraction.

Total RNA was isolated using TRIzol™ reagent (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically using a NanoDrop 2000 (Thermo Fisher Scientific) by measuring the A260/A280 ratio, and RNA integrity was verified on a 1% agarose gel. Complementary DNA (cDNA) was synthesized from 1µg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA).

qPCR was conducted on a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems) using SYBR Green Master Mix (Roche). Each 20µL reaction contained 10µL SYBR Green Master Mix, 1µL of each primer (forward and reverse), 2µL cDNA, and 6µL nuclease-free water. Amplification conditions were as follows: initial denaturation at 95°C for 10min, followed by 40 cycles of 95°C for 15s, 60°C for 30s, and 72°C for 30s.

The target genes analyzed were growth hormone (GH) and insulin-like growth factor 1 (IGF1), with β-actin used as the reference gene. Relative expression levels were calculated using the $2^{-}\Delta\Delta$Ct method **(Livak & Schmittgen, 2001)**. Primer sequences for all genes are listed in Table (2).

Table 2. Primer sequences used for gene expression analysis

Gene	Primer Sequences	Accession no.
GH	**F: CTGTCTGTCTGTCTGTCAGTCGT** **R: AGAGGAGACGCCCAAACAC**	**M97766.1**
IGF-1	**F: CCCGAACTTCCTCGACTTGA** **R: CCTCAGCCAGACAAGACAAAAA**	**AF033797**
β-actin	**F: ACCCACACAGTGCCCATC** **R: CAGGTCCAGACGCAGGAT**	**EF026001.1**

Calculated data

The gross energy (kcal/ kg DM) of experimental diets and body composition of tested group fish were calculated **(Blaxter, 1968; MacRae & Lobley, 2003)** using the following values, each g CP= 5.65 Kcal, g EE= 9.40 Kcal and g CF & NFE = 4.15 Kcal.

Statistical analysis

Collected data were subjected to statistical analysis as one way analysis of variance according to **SPSS (2020)**. Duncan's Multiple Range Test **(Duncan, 1955)** was used to separate means.

RESULTS

Chemical analysis of different experimental diets

As shown in Table (3), the proximate composition of black soldier fly larvae meal (BSFLM) consisted of 35.40% crude protein, 30.00% ether extract, 17.95% ash, and 5511 kcal/kg DM gross energy. The experimental diets were formulated to be iso-nitrogenous, with crude protein levels ranging narrowly between 29.26 and 29.88% across treatments (T1–T4). Crude fiber values remained stable (4.56–4.71%), whereas ether extract increased progressively with BSFLM inclusion, from 3.35% in the control diet (T1) to 10.80% in T4. Similarly, ash content rose from 9.11% (T1) to 16.80% (T4), while nitrogen-free extract (NFE) declined from 53.71% (T1) to 38.26% (T4). Gross energy values were comparable among diets, ranging from 4387 to 4461 kcal/kg DM.

These results confirm that BSFLM is a nutrient-dense protein and energy source, and that the formulated diets were nutritionally balanced, with minimal variation in protein and caloric content. Thus, observed differences among treatments can be attributed primarily to BSFLM inclusion rather than imbalances in dietary composition.

Table 3. Chemical analysis of different experimental diets

Item	**BSFLM**		**Experimental diets**		
		Zero BSFLM	**15% BSFLM**	**30% BSFLM**	**45% BSFLM**
		T_1	**T_2**	**T_3**	**T_4**
Moisture	**3.66**	**6.24**	**5.96**	**5.35**	**5.33**
Dry matter (DM)	**96.34**	**93.76**	**94.04**	**94.65**	**94.67**
Chemical analysis on DM basis					
Organic matter (OM)	**82.05**	**90.89**	**88.39**	**85.18**	**83.20**
Crude protein (CP)	**35.40**	**29.26**	**29.50**	**29.88**	**29.43**
Crude fiber (CF)	**8.31**	**4.57**	**4.56**	**4.45**	**4.71**
Ether extract (EE)	**30.00**	**3.35**	**5.69**	**8.13**	**10.80**
Nitrogen free extract (NFE)	**8.34**	**53.71**	**48.64**	**42.72**	**38.26**
Ash	**17.95**	**9.11**	**11.61**	**14.82**	**16.80**
Gross energy kcal/ kg DM	**5511**	**4387**	**4409**	**4410**	**4461**
Gross energy cal/ g DM	**5.511**	**4.387**	**4.409**	**4.410**	**4.461**

Gross energy (kcal/ kg DM) was calculated according to (**Blaxter1968; MacRae and Lobley 2003**).
Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.
BSFLM: black soldier fly larvae meal.

Performance and feed utilization

As presented in Table (4), dietary inclusion of BSFLM at 15, 30, and 45% significantly (P< 0.05) improved growth performance indices final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR) compared with the control group (T1). The best performance was achieved with the 15% BSFLM diet (T 2), followed by T 3 and T4. Survival remained 100% across all treatments, with no mortality observed.

Feed utilization parameters showed similar trends. Feed intake (FI) and crude protein intake (CPI) increased significantly in BSFLM-fed groups, with the highest values recorded in T2 (FI = 1133g; CPI = 334.24g). Feed conversion ratio (FCR) improved significantly ($P < 0.05$) in all BSFLM-fed groups relative to the control, with the most efficient conversion observed in T2 (4.569), compared to 9.229, 6.838, and 6.875 in T1, T3, and T4, respectively. Protein efficiency ratio (PER) also improved significantly ($P < 0.05$), with the highest values in T2, followed by T3 and T4.

In summary, dietary supplementation with BSFLM, particularly at 15%, enhanced growth performance, feed utilization, and protein efficiency in the Nile tilapia fingerlings.

Table 4. Performance and feed utilization of the Nile tilapia fingerlings fed diets contained different levels of black soldier fly larvae meal

Item	**Experimental diets**				**SEM**	**Sign. P<0.05**
	Zero BSFLM	**15% BSFLM**	**30% BSFLM**	**45% BSFLM**		
	T_1	**T_2**	**T_3**	**T_4**		
Growth performance						
Number of fish	**30**	**30**	**30**	**30**	**-**	**-**
IW, g	**363**	**367**	**366**	**364**	**0.685**	**NS**
FW, g	**472^c**	**615^a**	**520^b**	**516^b**	**15.780**	*****
TBWG, g	**109^c**	**248^a**	**154^b**	**152^b**	**15.384**	*****
Duration trial	**56 days**				**-**	**-**
ADG, g	**1.95^c**	**4.43^a**	**2.75^b**	**2.71^b**	**0.275**	*****
Feed utilization						
FI, g	**1006^c**	**1133^a**	**1053^b**	**1045^b**	**13.961**	*****
FCR	**9.229^c**	**4.569^a**	**6.838^b**	**6.875^b**	**0.508**	*****
FCP%	**29.26**	**29.50**	**29.88**	**29.43**	**-**	**-**
CPI, g	**294.36^d**	**334.24^a**	**314.64^b**	**307.54^c**	**4.359**	*****
PER	**0.3703^c**	**0.7420^a**	**0.4894^b**	**0.4942^b**	**0.041**	*****
Specific growth rate, relative growth rate, survival ratio and mortality rat %						
SGR	**0.21^c**	**0.40^a**	**0.27^b**	**0.27^b**	**0.021**	*****
RGR	**0.30^c**	**0.68^a**	**0.42^b**	**0.42^b**	**0.042**	*****
SR %	**100**	**100**	**100**	**100**	**-**	**-**
Mortality rate %	**Zero**	**Zero**	**Zero**	**Zero**	-	-

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).

SEM: Standard error of the mean, NS: Not significant. *: Significant at (P<0.05), IW: Initial weight, g. FW: Final weight, g, TBWG: Total body weight gain, g, ADG: Average daily gain, g, SGR: Specific growth rate, RGR: Relative growth rate, SR: Survival ratio, FI: Feed intake, FCR: Feed conversion ratio.

FCP%: Feed crude protein percentages, CPI: Crude protein intake, PER: Protein efficiency ratio.

Biochemical parameters of the different experimental groups

As shown in Table (5), serum biochemical profiles of the Nile tilapia were significantly (P< 0.05) influenced by BSFLM inclusion. Total protein and albumin levels increased in all BSFLM-fed groups, with the highest concentrations recorded in T2 (15%), followed by T3 (30%) and T4 (45%). Globulin levels also rose across treatments, though the increase was statistically significant only in T2. Liver enzyme activities (ALT and AST) and uric acid were reduced in fish fed the 15% BSFLM diet compared with the control, whereas higher inclusion levels (30% and 45%) resulted in increased values. Creatinine and glucose concentrations were significantly decreased (P< 0.05) in all BSFLM-fed groups relative to the control. Serum cholesterol remained unchanged in T2 but was significantly elevated in T3 and T4.

Table 5. Biochemical parameters of the Nile tilapia fingerlings fed diets containing different levels of black soldier fly larvae meal

Item	Experimental diets				SEM	Sign. P<0.05
	Zero BSFLM TG_1	15% BSFLM T_2	30% BSFLM T_3	45% BSFLM T_4		
Total Protein (g/dl)	**2.753^c**	**5.168^a**	**4.155^b**	**3.310^c**	**0.19**	*****
Albumin (g/dl)	**0.870^b**	**1.160^a**	**1.195^a**	**1.222^a**	**0.06**	*****
Globulin (g/dl)	**1.882^b**	**4.008^a**	**2.605^b**	**2.405^b**	**0.17**	*****
Glucose (mg/dl)	**115.3^a**	**92.31^b**	**97.39^b**	**101.3^{ab}**	**4.86**	*****
Total cholesterol (mg/dl)	**191.9^b**	**185.1^b**	**291.8^a**	**223.2^{ab}**	**5.26**	*****
Liver function						
ALT (Unit/l)	**109.3^{bc}**	**102.7^c**	**118.8^a**	**115.8^{ab}**	**2.38**	*****
AST (Unit/l)	**205.7^b**	**194.3^b**	**267.9^a**	**278.5^a**	**15.43**	*****
Kidney function						
Uric acid (mg/dl)	**17.62^b**	**10.87^c**	**18.31^b**	**23.92^a**	**0.39**	*****
Creatinine (mg/dl)	**26.05^a**	**21.63^c**	**23.03^b**	**16.09^d**	**0.56**	*****

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).

SEM: Standard error of the mean; *: Significant at P<0.05.

AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. BSFLM: black soldier fly larvae meal.

Gene expression

The influence of dietary black soldier fly (*Hermetia illucens*) larvae meal (BSFLM) on the hepatic expression of growth-related genes, insulin-like growth factor 1 (IGF1) and growth hormone (GH), in the Nile tilapia (*Oreochromis niloticus*) was evaluated using quantitative real-time PCR (Fig. 1). Fish were assigned to four dietary treatments: a control diet without BSFLM and three experimental diets in which fishmeal was partially replaced with BSFLM at 15% (150g/ kg), 30% (300g/ kg), and 45% (450g/ kg).

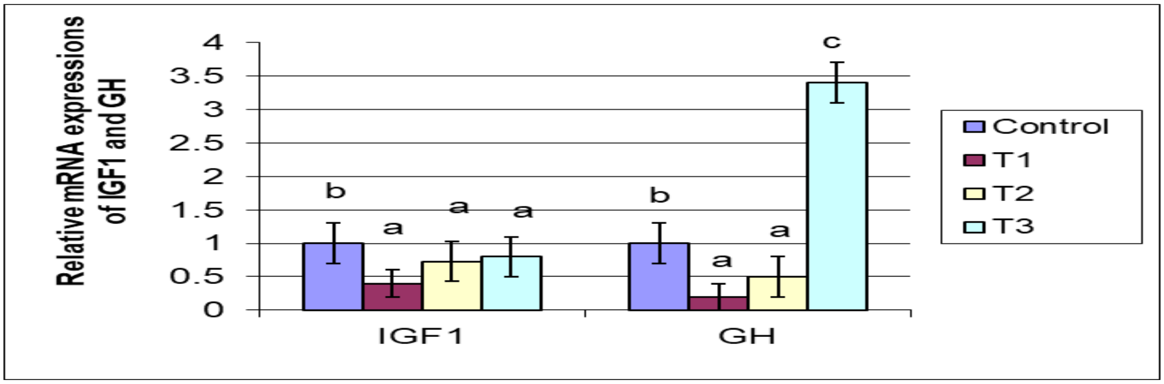

Fig. 1 Relative mRNA expression of IGF1, IGF2, and GH genes in liver tissues of the farmed Nile tilapia fish after feeding on diets with three different BSFL concentrations (15%, 30% and 45%). Different letters are significantly different at $P<0.05$.

IGF1 gene expression

Relative mRNA expression of IGF1 in the liver of the Nile tilapia did not exhibit significant enhancement with increasing levels of BSFLM supplementation (Fig. 1). The control group and the 15% BSFLM inclusion group (T1) recorded the highest IGF1 expression levels, whereas fish fed 30% (T2) and 45% (T3) diets showed comparatively lower expression. Statistical analysis indicated that the control group displayed significantly higher ($P< 0.05$) IGF1 expression than all BSFLM-fed groups, while no significant differences were detected among T1, T2, and T3.

GH gene expression

In contrast, hepatic GH expression exhibited a more pronounced response to BSFLM inclusion (Fig. 1). The control and 15% BSFLM inclusion group (T1) showed moderate expression levels, whereas a significant ($P < 0.05$) downregulation was detected in fish fed the 30% BSFLM diet (T2). Interestingly, fish receiving the 45% BSFLM diet (T3) displayed a marked upregulation, with GH expression increasing approximately 3.5-fold relative to the control. This upregulation in T3 was statistically significant ($P< 0.05$) compared with all other dietary groups.

Relative mRNA expression of IGF1 and GH in the liver of the Nile tilapia fed BSFLM-based diets is shown in Fig. (1). IGF1 expression did not exhibit significant enhancement with increasing levels of BSFLM supplementation. The control group and the 15% inclusion (T1) recorded the highest IGF1 levels, whereas fish fed 30% (T2) and 45% (T3) diets showed comparatively lower expression. Statistical analysis revealed that the control group was significantly ($P< 0.05$) higher than all BSFLM-fed groups, with no significant differences observed among T1, T2, and T3. In contrast, GH expression demonstrated a more pronounced dietary response. While the control and T1 groups maintained moderate levels, a significant ($P< 0.05$) downregulation was detected in fish fed the 30% diet (T2). Notably, the 45% inclusion group (T3) exhibited a sharp upregulation, with GH expression increasing approximately 3.5-fold relative to the control, representing a statistically significant difference ($P< 0.05$) compared to all other treatments.

DISCUSSION

The proximate analysis of BSFLM used in this study confirmed its high nutritional value, containing 35.40% crude protein, 30.00% ether extract, 17.95% ash, and 5511 kcal/kg DM. This composition highlights BSFLM as a rich source of protein and energy, making it a suitable alternative to conventional aquafeed ingredients. The experimental diets were iso-nitrogenous (29.26–29.88% CP) and exhibited minimal variation in caloric content (4387–4461 kcal/kg DM), ensuring that observed differences in fish performance were primarily attributable to BSFLM inclusion rather than nutrient imbalance **(Abozaid *et al.*, 2025)**. These findings are consistent with previous studies emphasizing the nutritional potential of insect meals as sustainable protein sources **(Tacon & Metian, 2008; Obirikorang *et al.*, 2015; Kishawy *et al.*, 2022)**. BSFLM's high protein content and favorable amino acid profile make it a promising feed ingredient across aquaculture species. Positive growth and physiological outcomes have been reported in Pacific white shrimp **(Cummins *et al.*, 2017)**, rainbow trout **(Renna *et al.*, 2017)**, the Nile tilapia **(Fitches & Smith, 2018)** and Atlantic salmon **(Belghit *et al.*, 2019)**. In the present study, the comparable protein levels across diets ensured that differences in growth and feed utilization were largely due to the nutritional and bioactive properties of BSFLM rather than variations in diet formulation.

Dietary inclusion of BSFLM at 15, 30, and 45% significantly enhanced growth performance in the Nile tilapia fingerlings, including final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and relative growth rate (RGR), compared to the control. The 15% inclusion level (T2) consistently yielded the best results, suggesting that moderate supplementation provides an optimal balance between nutrient availability and digestibility. Survival was 100% across all treatments, indicating good palatability and no adverse effects on fish health. Feed utilization metrics further support the beneficial role of BSFLM. Fish fed BSFLM-based diets exhibited higher feed intake (FI) and crude protein intake (CPI), with the highest values recorded in T2 (FI: 1133 g; CPI: 334.24 g). Feed conversion ratio (FCR) improved significantly in BSFLM-fed groups, with the most efficient conversion observed in T2 (4.569) compared to the control (9.229). Similarly, protein efficiency ratio (PER) was higher in BSFLM-supplemented diets, reflecting enhanced dietary protein utilization. These outcomes are in line with previous reports indicating that partial or full replacement of fishmeal with BSFLM supports growth and feed efficiency without compromising fish health **(Belghit *et al.*, 2019; Tippayadara *et al.*, 2021; Kishawy *et al.*, 2022)**. The present findings are consistent with previous studies demonstrating that partial or complete replacement of fishmeal with BSFLM does not compromise growth performance across diverse aquaculture species. For example, **Kishawy *et al.* (2022)** reported that replacing fishmeal with partially defatted BSFLM up to 100% had no adverse effect on body weight gain, feed conversion ratio (FCR), or specific growth rate (SGR) in the Nile tilapia, and all experimental diets were readily accepted by the fish. Similar outcomes have been observed in the Pacific white shrimp **(Cummins *et al.*, 2017)**, rainbow trout **(Renna *et al.*, 2017)**, Atlantic salmon **(Belghit *et al.*, 2019)**, Japanese sea bass **(Wang *et al.*, 2019)**, European sea bass **(Abdel-Tawwab *et al.*, 2020)**, Siberian sturgeon **(Caimi *et al.*, 2020)**, and the Nile tilapia **(Tippayadara *et al.*, 2021)**. **Devic *et al*. (2018)** further demonstrated that replacing up to 80% of fishmeal with BSFLM did not negatively affect final weight, FCR, or survival in the Nile tilapia. The observed improvements in growth and feed utilization in the present study can be attributed to the favorable nutritional profile of BSFLM, including its high protein content, lipid fraction, and bioactive compounds, which collectively enhance feed palatability, digestion, and nutrient assimilation. **Rawski *et al.* (2020)** similarly reported that BSFLM supplementation in Siberian sturgeon diets improved weight gain, SGR, and feed efficiency while reducing dependence on fishmeal and fish oil. Feed acceptance in this study was highest with diets containing 10–30% BSFLM, confirming that insect-based feeds are palatable and readily consumed by the Nile tilapia. Significant enhancements in FW, TBWG, ADG, SGR, and RGR were particularly pronounced at 15% inclusion, highlighting the functional role of insect protein in promoting growth. While feed intake was generally comparable among treatments, both FCR and protein efficiency ratio (PER) improved in BSFLM-fed groups, with optimal responses typically observed at lower inclusion levels (5–15%). The beneficial effects of BSFLM on growth and feed utilization may also reflect its bioactive components, such as antimicrobial peptides (AMPs), lauric acid, and chitin with prebiotic properties, which enhance nutrient assimilation and support immune function **(Park *et al.*, 2015; Kasumyan, 2016; Lee *et al.*, 2020; Moretta *et al.*, 2020)**. Moreover, full-fat BSFLM provides both nutritional efficiency and environmental sustainability, maximizing the potential of *Hermetia illucens* biomass without the negative impacts associated with excessive chitin levels observed in some defatted products **(Rawski *et al.*, 2020)**. Biochemical parameters provide valuable insight into the physiological status of fish and the safety of dietary interventions. In the present study, moderate inclusion of BSFLM (15%) significantly increased serum protein, albumin, and globulin levels in the Nile tilapia. These enhancements likely reflect the high content of essential amino acids, including methionine, lysine, threonine, isoleucine, leucine, and valine, which support protein metabolism and immune function **(Oteri *et al.*, 2021)**. Similar observations have been reported in red hybrid tilapia **(Aisyah *et al.*, 2022)** and the Nile tilapia fed defatted

BSFLM **(Kishawy *et al.*, 2022)**, where blood profiles indicated good health status. Moreover, fish receiving 15% BSFLM exhibited reduced levels of ALT, AST, cholesterol, glucose, creatinine, and uric acid, demonstrating the safety of moderate BSFLM inclusion for maintaining hepatic and renal function. Comparable hepatoprotective effects have been observed in Atlantic salmon **(Belghit *et al.*, 2019)** and goldfish **(Khieokhajonkhet *et al.*, 2022)**, whereas higher inclusion levels in the present study led to significant elevations in liver enzymes and metabolites, indicating potential metabolic stress at excessive doses. These findings are consistent with previous research showing that insect meals maintain normal serum biochemistry, absence of tissue inflammation, and overall fish health **(Fazio, 2019; Dawood *et al.*, 2020; Caimi *et al.*, 2021)**. Overall, moderate dietary inclusion of BSFLM (~15%) supports optimal growth, efficient feed utilization, and safe physiological responses, whereas excessive inclusion may impose metabolic challenges. At the molecular level, BSFLM inclusion influenced the expression of growth-related genes. Growth hormone (GH) was strongly upregulated at the highest inclusion level (45%), whereas insulin-like growth factor 1 (IGF1) showed a slight downward trend at elevated levels. This non-linear, dose-dependent response suggests that BSFLM modulates the GH/IGF1 axis in a complex manner. The substantial GH upregulation at 45% may be attributed to the high levels of essential amino acids, bioactive lipids such as lauric acid, and micronutrients in BSFL, which can stimulate endocrine pathways involved in growth regulation **(Makkar *et al.*, 2014; Gasco *et al.*, 2019)**. Although IGF1 expression did not increase in parallel with GH, such uncoupled responses are not uncommon in fish. The GH/IGF1 axis is subject to complex feedback regulation, with IGF1 transcription influenced by factors beyond GH, including insulin, nutritional status, and cortisol **(Reindl & Sheridan, 2012)**. High BSFL inclusion may trigger GH induction as a compensatory mechanism, while IGF1 expression is moderated by homeostatic feedback or hepatic sensitivity, potentially linked to nutrient overload or metabolic stress. Evidence from other species supports this dose- and species-dependent modulation: in zebrafish, total replacement of fishmeal with BSFL altered IGF1 and muscle-regulating gene expression without impairing growth **(Li *et al.*, 2021)**. The strong GH induction observed in tilapia at 45% inclusion may enhance hypertrophic growth potential, but the lack of a corresponding IGF1 response raises questions regarding the efficiency and sustainability of growth at high inclusion levels.

Previous research indicates that moderate BSFLM inclusion supports growth performance without negatively affecting feed efficiency or fish health **(Belghit *et al.*, 2019; Gasco *et al.*, 2019)**. In the present study, the transcriptional dysregulation observed at higher inclusion levels may reflect a physiological threshold beyond which metabolic stress outweighs nutritional benefits. This underscores the importance of identifying optimal inclusion rates that maximize growth responses while maintaining endocrine and metabolic balance. Future studies should integrate gene expression data with circulating GH and IGF1 protein concentrations, growth performance metrics (e.g., weight gain, feed conversion ratio), and physiological biomarkers to clarify the biological significance of these transcriptional changes. Additionally, evaluating intermediate BSFLM inclusion levels could help define the dietary threshold that ensures endocrine stability while fully exploiting the growth-promoting potential of this sustainable protein source.

CONCLUSION

The findings of the present study demonstrate that black soldier fly larvae meal (BSFLM) can be incorporated into the Nile tilapia diets as a sustainable alternative to fishmeal, supporting growth performance, feed utilization, and physiological health. Moderate inclusion levels (15–30%) improved feed acceptance, growth indices, and serum biochemical parameters, confirming the nutritional value and safety of BSFLM. At the molecular level, BSFLM supplementation significantly modulated growth-related gene expression, with GH showing strong upregulation at 45% inclusion, while IGF1 expression exhibited a downward trend. These results indicate that although high BSFLM levels may stimulate endocrine responses, moderate inclusion rates are more effective in maintaining a balanced growth-promoting environment without potential metabolic stress. Collectively, BSFLM represents a promising and environmentally sustainable protein source for aquafeeds; however, further studies integrating gene expression, circulating hormone levels, growth performance, and long-term health effects are recommended to determine optimal inclusion thresholds.

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