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Effect of Replacing Dietary Soybean Meal with *Galleria mellonella* Larvae Powder on Growth Performance of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The study was conducted to assess the effects of using *Galleria mellonella* larvae (GML) as an unconventional protein source (insect protein) replacing soybean meal (SBM) in the Nile tilapia diets. Approximatley 180 fish (with an initial average body weight of 5.6±0.18g) were randomly assigned among 12 experimental aquariums, with 15 fish per aquarium, giving three replicates of 45 fish for each treatment. GML replaced 0, 5, 10, and 15% of the soybean meal, which constituted 40% of the control diet. These replacements were corresponding to 0, 20, 40, and 60g/ kg for diets D1, D2, D3, and D4, respectively, for 56 days. All test diets were designed to be iso-nitrogenous. At the end of the experiment, growth productivity, feed utilization, body composition, biochemical analysis, immune parameters and economical evaluation were examined. The results indicated that, metrics such as FW, TBWG, ADG, SGR, and SR showed improvements in diets D2, D3, and D4, with a mortality rate of 6.67% in the control group (D1) and 0% in the other treatments. Fish fed on GML diets D2, D3, and D4 demonstrated a significant ($P< 0.05$) increase in FI, FCR, CPI, PER, energy retention percentage and lysozyme activity in all treatment groups compared to the control, whereas there were non-significant changes ($P> 0.05$) in ALT, AST, glucose, and cholesterol. Additionally, feed formulation costs decreased, with net improvements in the feeding cost percentages of 6.17, 8.70, and 10.07% for diets D2, D3, and D4, respectively, compared to the control group. The study revealed that using GML as an insect protein holds great potential as a substitute for SBM feeds to increase the production of the Nile tilapia.

INTRODUCTION

The Nile tilapia is considered as one of the most cultivated species due to its high adaptability to various environmental conditions, rapid growth rate, and ability to thrive on a wide range of diets **(Munguti *et al.*, 2022)**. This species is particularly important in many developing countries, where it is a crucial animal protein source and income **(Boyd *et al.*, 2022)**. As the demand for fish continues to rise globally, driven by population growth and changing dietary preferences, the sustainable production of the Nile tilapia has become increasingly essential **(Tran *et al.*, 2022)**. However, a major challenge faced by the aquaculture industry is the high cost of fish diets, which is the largest expenditure in semi-intensive and intensive fish farming **(Munguti *et al.*, 2021)**. This high cost is mainly due to the animal protein content that is used in the diet formulation, which constitute the largest proportion of the commercial fish feed **(Hardy, 2010)**. New aquaculture technologies, farming systems, productivity improvements, and strategies for controlling production costs are required to sustain advancements in aquaculture **(El-Sayed, 2021; Fayed *et al.*, 2023)**. Over the last decades, the production of fish meal and fish oil has been estimated at about 6.5 and 1.3 million metric tons, respectively, with fluctuating production levels. Additionally, increasing in human populations along with changing quality food perceptions led to an increased demand for fish as an affordable animal protein source. Unless the fish supply is increased through sustainable productions, fish protein would be scarce and would become expensive commodities according to **FAO (2012)**.

To reduce diet costs, researchers are exploring plant proteins as substitutes for animal proteins in fish feed **(El-Saidy & Gaber, 2003)**. The immune and performance status of cultured fish can be improved by producing diets which should be economical viable and eco-friendly **(Ataguba *et al.*, 2017; Hassaan *et al.*, 2019; Saleh *et al.*, 2020; Wassef *et al.*, 2020)**. Recently, insects have recently been considered as a sustainable resource for aqua-feed production **(Henry *et al.*, 2018; Nesic & Zagon, 2019)**. Although, insect production has not yet reached a scale where it can compete with other protein sources such as fish meal and vegetable protein supplies, it benefits the environment, particularly in terms of energy use **(Thévenot *et al.*, 2018; Sogari *et al.*, 2019)**.

Black soldier fly larvae (BSFL) are considered to be one of the most promising insect species for replacing traditional proteins, offering an environmentally friendly alternative **(Lu *et al.*, 2022)**. They have a high protein content, ranging from 31 to 59%, with an amino acid composition similar to fish meal and superior to soybean **(Barroso *et al.*, 2014; Nogales-Mérida *et al.*, 2019)**. Additionally, BSFL are a rich source of minerals, particularly calcium and phosphorus, which range from 11 to 49% **(Makkar *et al.*, 2014; Nogales-Mérida *et al.*, 2019)**. BSFL have been successfully used in fish diets for species like the red sea bream **(Ido *et al.*, 2019)**, the rainbow trout (*Oncorhynchus mykiss*) **(Józefiak *et al.*, 2019)**, *Siberian sturgeon* **(Rema *et al.*, 2019)**, and the Atlantic salmon **(Li *et al.*, 2020)**. Therefore, **Priyadarshana *et al.* (2022)** demonstrated that insect protein could replace up to 50% of fish meal and other protein sources like soybean meal in tilapia diets, with favorable outcomes. They also highlighted the potential of BSFL as a fish meal replacement, noting significant variations among species. Therefore, this study aimed to investigate the impact of replacing soybean meal with *Galleria mellonella* larvae (GML) that are used as a traditional source of protein on the growth productivity, feed utilization, body composition, biochemical analysis and immune status of the Nile tilapia, as well as evaluating the economic viability of this replacement.

MATERIALS AND METHODS

Experimental design

A total of 180 fish were acclimated for 15 days before being randomly assigned to 12 aquariums, each with a capacity of 60 liters and dimensions of 80 × 40 × 30cm. The initial body weight of the fish was 84± 0.369g. Three aquariums represented one treatment, with each aquarium containing 15 fish.

Galleria mellanella larvae source

Stock culture of *Galleria mellonella* larvae (GML) was collected from old black combs removed by a beekeeper. The infested old black combs were obtained from the bee apiary of the Department of Pests and Plant Production at the National Research Center farm in Noubaria. The collected combs were examined to obtain the 6th instar larvae of the greater wax moth, which were then stored in a refrigerator. The remaining wax was placed in glass cages (60 × 60 × 30 cm) until the eggs hatched or the small larvae reached the last instar, after which they were collected. These larvae were dried in an oven at 60°C for 15 minutes before use.

Experimental diets

Galleria mellonella larvae (GML) were used as an unconventional protein source to replace soybean meal at four different levels: 0, 5, 10, and 15% of soybean meal, which made up 40% of the basal diet. These levels of GML corresponded to 0, 2, 4, and 6% of the total diet composition corresponding to 0, 20, 40, and 60g/ kg of diet for D1, D2, D3, and D4, respectively, as detailed in Table (1). The experimental diets were administered continuously for 56 days, with hand-feeding taking place over this period, from mid-December 2023 to mid-February 2024.

Table 1. Composition of the different experimental diets

Ingredients	**Experimental diets**				**Price of tone LE**
	Control Zero GML	**Replacing 5% of SBM by GML**	**Replacing 10% of SBM by GML**	**Replacing 15% of SBM by GML**	
	D_1	**D_2**	**D_3**	**D_4**	
Composition of tested diets					
Galleria mellonella larvae (GML)	0.00	2.00	4.00	6.00	15000
Soybean meal (SBM), (44% CP)	40.00	38.00	36..00	34.00	33000
Concentration (56% CP)	17.00	17.00	17.00	17.00	25000
Ground yellow corn (8% CP)	28.00	28.00	28.00	28.00	12500
Wheat bran (13% CP)	10.00	10.00	10.00	10.00	14500
Vegetable oil	3.00	3.00	3.00	3.00	50000
Vitamins and minerals mixture**	2.00	2.00	2.00	2.00	40000
Price of ton fed (LE)	24700	24340	23980	23620	---
Price of kg fed (LE)	24.700	24.340	23.980	23.620	---

SBM: Soybean meal. GML: *Galleria mellonella* larvae. ** 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). Raanan Aqua Feed Px, Maridav Ghana Limited.

Parameters of growth performance

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)]

Protein productive value (PPV %) = [PR_1– PR_0 / PI] 100.

Where, PR_1 = Total fish body protein at the end of the experiment.

PR_0 = Total fish body protein at the start of the experiment.

PI = Protein intake.

Energy retention percentages (ER %)

The energy retention percentage was calculated according to the following equation:

Energy retention (ER %) = E-E_0 / E_F X 100

Where, E= Energy in fish carcass (kcal) at the end of the experiment.
E_0= Energy in fish carcass (kcal) at the start of the experiment.
E_F = Energy (kcal) in feed intake.

Estimated blood parameters

Blood samples were collected from the caudal vein of 5 fish (killed by an overdose of anesthetic, i.e. 3-amino benzoic acid ethyl ester; Sigma-Aldrich, Basingstoke, U.K.) by insulin syringe and left to clot for 2h, prior to centrifugation (1600g, 25min, 4°C). Serum samples were collected and stored at -20°C until used.

Immune parameters

Lysozyme activity

The turbidimetric assay for lysozyme was carried out according to the method of **Parry *et al*. (1965)**.

Total protein, albumin and globulin

Total protein **(Cannon, 1974)** and albumin **(Tietz, 1995)** were estimated using commercial biochemical kits (Bio-diagnostics, Egypt). Globulin was obtained by subtracting albumin concentration from total protein concentration. Each biochemical parameter was colorimetrically analyzed according to its manufacturer's instructions using an Agilent Cary UV-Vis spectrophotometer.

Biochemical assays

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) **(Reitman & Frankel, 1957)**, cholesterol **(Ellefson & Caraway, 1976)** and glucose concentrations **(Caraway & Watts, 1987)**, and uric acid **(Tietz, 1990)** were also determined colorimetrically in serum samples using ordinary commercial kits (Bio-diagnostics, Giza, EGYPT), and following the manufacturer's instruction for each parameter using an Agilent Cary UV-Vis spectrophotometer (100/300 Series).

Body composition

At the beginning, 20 fish were utilized, while at the end, nine fish from each treatment were randomly selected to assess their whole body composition. The analysis of the tested diets and fish body composition was conducted following the **AOAC (2016)** methods.

Calculated data

The gross energy (kcal/ kg DM) of experimental diets and body composition of tested group fish were calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)** and protein energy ratio (mg CP/ Kcal ME) calculated according to **NRC (2011)**.

Statistical analysis

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

RESULTS

Chemical analysis of the experimental diets

Our results in Table (2) indicate that *Galleria mellonella* larvae (GML) are a rich source of nutrients, with a crude protein (CP) content of 38.5%, an ether extract (EE) content of 50.71%, a gross energy value of 7346 kcal/kg DM, a metabolizable energy value of 600.25kcal/ kg DM, and a protein energy ratio of 64.14mg CP/kcal ME. Alternatively, the CP% ranged from 29.48 to 29.92%. The gross energy (GE) values ranged from 4412 to 4715, while the metabolizable energy (ME) values were between 339.25 and 359.49. Additionally, the protein energy ratio varied from 83.23 to 86.89mg CP/kcal ME among the four experimental diets. These values are considered sufficient to meet the requirements of the Nile tilapia fish. Although all tested rations appeared to be iso-nitrogenous, they differed in their calorie and energy content, which can be attributed to the high ether extract (EE) or GE content in GML.

Table 2. Chemical analysis of *Galleria mellonella* larvae and the different experimental diets

Ingredients	**GML**	**Experimental diets**			
		Control Zero GML	**Replacing 5% of SBM by GML**	**Replacing 10% of SBM by GML**	**Replacing 15% of SBM by GML**
		D_1	**D_2**	**D_3**	**D_4**
Moisture	65.00	7.66	7.46	7.07	6.89
Dry matter (DM)	35.00	92.34	92.54	92.93	93.11
Chemical analysis on DM basis					
Organic matter (OM)	98.94	89.33	89.78	89.87	89.98
Crude protein (CP)	38.50	29.48	29.85	29.92	29.21
Crude fiber (CF)	5.80	7.32	7.22	7.14	7.09
Ether extract (EE)	50.71	5.00	6.96	8.70	10.33
Nitrogen free extract (NFE)	3.93	47.53	45.75	44.11	43.35
Ash	1.06	10.67	10.22	10.13	10.02
Gross energy kcal/ kg DM	7346	4412	4539	4635	4715
Gross energy cal/ g DM	7.346	4.412	4.539	4.635	4.715
Metabolizable energy kcal/ kg DM	600.25	339.29	350.72	359.49	366.93
Protein energy ratio (mg CP/ Kcal ME)	64.14	86.89	85.11	83.23	79.61

SBM: Soybean meal. GML: *Galleria mellonella* larvae. Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal. Metabolizable energy (ME): calculated using values of 4.50, 8.15 and 3.49 Kcal for protein, fat and carbohydrate, respectively, calculated according to **NRC (2011)**. Protein energy ratio (mg CP/ Kcal ME): Calculated according to **NRC (2011)**.

Growth and survival rate

The data in Table (3) demonstrate that the values for final weight (FW), total body weight gain (TBWG), average daily gain (ADG), specific growth rate (SGR), and survival rate (SR) improved when fish were fed diets where 5, 10, and 15% of soybean meal (SBM) in the control diet were replaced with GML. Moreover, the mortality rate was 6.67% in the control group (D1), but it was zero in the other groups (D2, D3, and D4). Overall, the inclusion of GML in the diet did not have a significant impact on the mentioned parameters.

Table 3. Growth performance, specific growth rate and survival ratio of different experimental groups

Item	Experimental diets				SEM	Sign. *P*<0.05
	Control Zero GML	Replacing 5% of SBM by GML	Replacing 10% of SBM by GML	Replacing 15% of SBM by GML		
	D_1	D_2	D_3	D_4		
Initial weight, g (IW)/15 fish	85	84	83	84	0.369	NS
Final weight, g (FW)/15 fish	340^b	362^a	364^a	370^a	3.633	*
Total body weight gain, g (TBWG)	255^b	278^a	281^a	286^a	3.794	*
Duration experimental period			**56 days**			
Average daily gain, g (ADG)	4.55^b	4.96^a	5.02^a	5.11^a	0.068	*
Specific growth rate (SGR)	1.08^b	1.14^a	1.16^a	1.15^a	0.010	*
Number of fish at the starter	45	45	45	45	-	-
Number of fish at the end	42	45	45	45	-	-
Survival ratio (SR)	93.33	100	100	100	1.196	NS
Number of dead fish	3	Zero	Zero	Zero	-	-
Mortality rate percentages	6.67	Zero	Zero	Zero	1.196	NS

SBM: Soybean meal. GML: *Galleria mellonella* larvae. a and b: Means in the same row having different superscripts differ significantly ($P< 0.05$). SEM: Standard error of mean. NS: Not significant. *: Significant at ($P< 0.05$).

Feed utilization of the different experimental groups

The data in Table (4) reveal that feed intake (FI), feed conversion ratio (FCR), crude protein intake (CPI), and protein efficiency ratio (PER) were significantly increased ($P< 0.05$) when 5, 10, and 15% of the soybean meal (SBM) in the control diet (40%) were replaced with GML in the tested diets for the Nile tilapia fish.

Table 4. Feed utilization of the different experimental groups

Item	Experimental diets				SEM	Sign. *P*<0.05
	Control Zero GML	Replacing 5% of SBM by GML	Replacing 10% of SBM by GML	Replacing 15% of SBM by GML		
	D_1	D_2	D_3	D_4		
Total body weight gain, g (TBWG)	255^b	278^a	281^a	286^a	3.794	*
Feed intake (FI), g	427.56^c	445.20^{ab}	443.10^b	451.92^a	2.888	*
Feed conversion ratio (FCR)	1.68^b	1.60^a	1.58^a	1.58^a	0.013	*
Feed crude protein %	29.48	29.85	29.92	29.21	-	-
Crude protein intake (CPI), g	126.04^b	132.89^a	132.58^a	132.01^a	0.904	*
Protein efficiency ratio (PER)	2.023^c	2.092^c	2.119^b	2.167^a	0.017	*

SBM: Soybean meal. GML: *Galleria mellonella* larvae. a, b and c: Means in the same row having different superscripts differ significantly (*P*<0.05). SEM: Standard error of mean. *: Significant at (*P*<0.05). FCR: Expressed as g of DM intake/g gain. PER: Expressed as g of gain/g CP intake.

Blood parameters of the different experimental groups

Immunological parameters

All fish groups that fed diets containing GML larvae exhibited the highest significant lysozyme activity compared to the control (*P*< 0.05), especially, the dose of 5% which recorded the highest activity (Fig. 1). Similarly, the highest total protein values (Fig. 2) were reported in fish groups fed with GML compared to the control, particularly in 5% dose (showed the highest value), but without a significant difference. Additionally, the fish group fed with 5% of *Galleria mellonella* larvae revealed the highest significant albumin value (*P*< 0.05) compared to the control and other groups (Fig. 3). On the contrary, the highest globulin value was recorded in fish fed 15% of GML compared to other groups (Fig. 4).

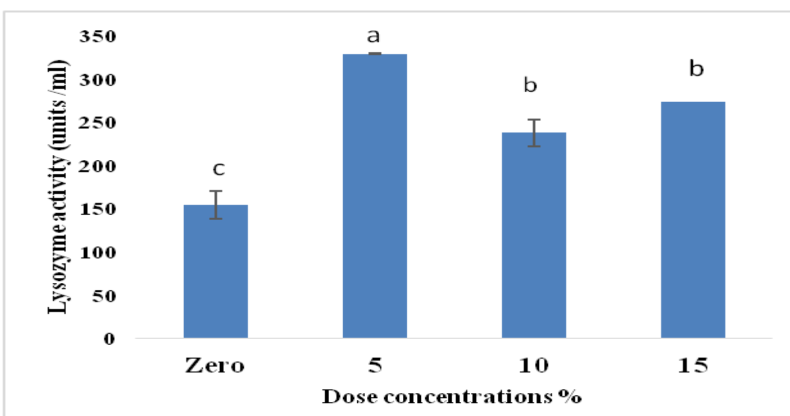

Fig. 1. Lysozyme activity in the Nile tilapia fed diets with 5, 10, and 15% replacement of soybean meal (SBM) with *Galleria mellonella* larvae (GML) was compared to a control diet containing 40% SBM. Groups labeled with the same letter are not statistically different (*P*> 0.05). The bars represent the mean values, with standard errors indicated.

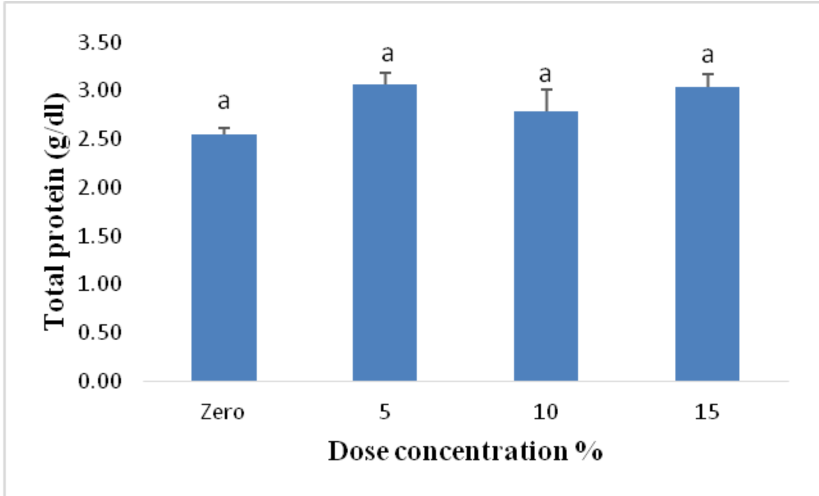

Fig. 2. Total protein of the Nile tilapia fed diets with 5, 10 and 15% replacement of soybean meal (SBM) with *Galleria mellonella* larvae (GML) were compared to a control diet containing 40% SBM. Groups labeled with the same letter are not statistically different (*P*> 0.05). The bars represent the mean values with standard error indicated.

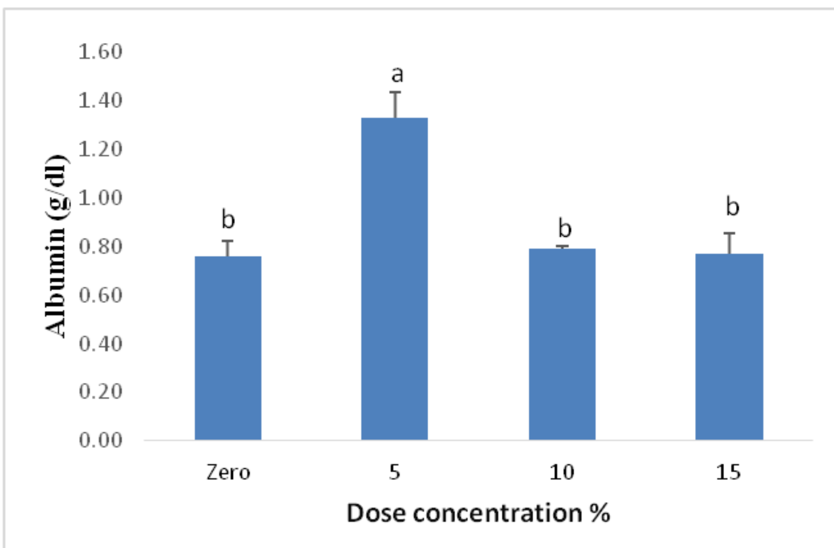

Fig. 3. Albumin levels in the Nile tilapia fed diets with 5, 10, and 15% replacement of soybean meal (SBM) with *Galleria mellonella* larvae (GML) were compared to a control diet containing 40% SBM. Groups labeled with the same letter are not statistically different (*P* > 0.05). The bars represent the mean values, with standard errors indicated.

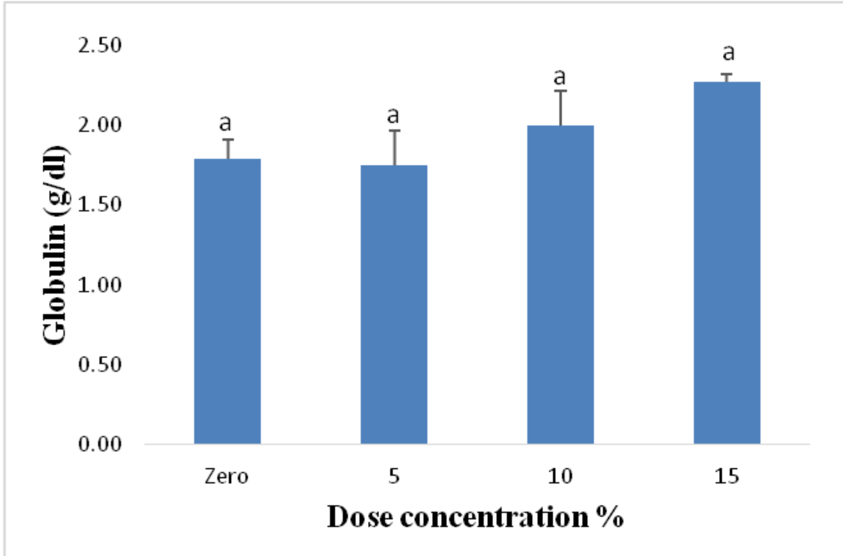

Fig. 4: Globulin levels in Nile tilapia fed diets with 5, 10, and 15% replacement of soybean meal (SBM) with *Galleria mellonella* larvae (GML) were compared to a control diet containing 40% SBM. Groups labeled with the same letter are not statistically different (*P*> 0.05). The bars represent the mean values with standard errors indicated.

Biochemical parameters

The data in Table (5) demonstrate that incorporating GML into fish diets caused non-significant changes (*P*> 0.05) in most of the biochemical parameters. The levels of serum AST, ALT, glucose, and cholesterol were insignificantly (*P*> 0.05) affected in all the treated groups compared to the control one. On the other hand, uric acid concentration showed its highest significant value (*P*< 0.05) in fish fed a diet with 15% GML group, followed by G10 and G5 compared to the control group.

Table 5. Biochemical parameters of the different experimental groups

Item	Experimental diets				SEM	Sign. $P<0.05$
	Control Zero GML	Replacing 5% of SBM by GML	Replacing 10% of SBM by GML	Replacing 15% of SBM by GML		
	D_1	D_2	D_3	D_4		
AST (Unit/l)	203.78[a]	231.24[a]	215.12[a]	266.03[a]	17.93	NS
ALT (Unit/l)	57.19[a]	58.54[a]	58.51[a]	64.88[a]	3.90	NS
Glucose (mg/dl)	71.56[a]	81.74[a]	95.94[a]	79.37[a]	6.39	NS
Cholesterol (mg/dl)	126.90[a]	124.75[a]	135.34[a]	121.60[a]	16.13	NS
Uric acid (mg/dl)	15.91[c]	14.39[c]	19.30[b]	22.68[a]	1.42	*

SBM: Soybean meal. GML: *Galleria mellonella* larvae. a, b, c and d: Means in the same row having different superscripts differ significantly ($P< 0.05$). SEM: Standard error of mean. *: Significant at ($P< 0.05$). NS: Not significant. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase.

Fish body composition of different experimental groups

The data in Table (6) show that the Nile tilapia fish that fed diets containing GML resulted in a significant ($P< 0.05$) increase in the body composition parameters, including dry matter (DM), organic matter (OM), ether extract (EE), and gross energy content. Meanwhile, there was a significant ($P< 0.05$) decrease in the values of moisture, crude protein, and ash content compared to the control.

Table 6. Fish body composition of initial and different experimental groups fed tested diets

Item	Body composition of initial fish	Experimental diets				SEM	Sign. $P<0.05$
		Control Zero GML	Replacing 5% of SBM by GML	Replacing 10% of SBM by GML	Replacing 15% of SBM by GML		
		D_1	D_2	D_3	D_4		
Moisture	80.41	76.52[a]	74.48[b]	73.36[c]	73.61[c]	0.379	*
Dry matter (DM)	19.59	23.48[c]	25.53[b]	26.64[a]	26.39[a]	0.379	*
Chemical analysis on DM basis							
Organic matter (OM)	82.04	85.33[b]	85.70[b]	88.16[a]	88.28[a]	0.418	*
Crude protein (CP)	65.56	60.93[a]	58.09[c]	59.04[b]	55.19[d]	0.631	*
Ether extract (EE)	16.48	24.40[d]	27.61[c]	29.12[b]	33.09[a]	0.942	*
Ash	17.96	14.67[a]	14.30[a]	11.84[b]	11.72[b]	0.418	*
Gross energy kcal/ 100g	525.33	573.61[c]	587.74[c]	607.30[b]	622.87[a]	5.666	*
Gross energy cal/ g DM	5.2533	5.7361[d]	5.8774[c]	6.0730[b]	6.2287[a]	0.057	*

SBM: Soybean meal. GML: *Galleria mellonella* larvae. a, b, c and d: Means in the same row having different superscripts differ significantly (P< 0.05). SEM: Standard error of mean. *: Significant at ($P< 0.05$). Gross energy (kcal/ kg DM) was calculated according to **Blaxter (1968)** and **MacRae and Lobley (2003)**. Where, each g CP = 5.65 Kcal, g EE = 9.40 kcal and g CF and NFE = 4.15 Kcal.

Energy retention and protein productive value percentages

Results of Table (7) reveal that including GML in the diets of the Nile tilapia at levels of 5, 10 and 15% (D_2, D_3 and D_4, respectively) significantly (P< 0.05) increased the energy retention (ER)% compared to the control (D_1). Specifically, the feed conversion ratio (ER%) improved by 4.74%, 8.45%, and 9.76% for fish fed diets with 5%, 10%, and 15% replacement of soybean meal (SBM) with *Galleria mellonella* larvae (GML), respectively. In contrast, the protein productive value (PPV%) significantly decreased (P< 0.05) in fish fed the diet containing 15% GML (D4). There was no significant effect (P> 0.05) on PPV% for fish fed diets containing 5% or 10% GML (D2 and D3, respectively) compared to the control. PPV% improved by 0.77% in the D3 group but decreased by 2.78% and 5.97% in the D2 and D4 groups, respectively, compared to the control group (D1).

Table 7. Energy retention (ER) and protein productive value (PPV) % of different experimental groups

Item	**Experimental diets**				**SEM**	**Sign. *P*<0.05**
	Control Zero GML	**Replacing 5% of SBM by GML**	**Replacing 10% of SBM by GML**	**Replacing 15% of SBM by GML**		
	D_1	**D_2**	**D_3**	**D_4**		
Initial weight (IW), g	85	84	83	84	0.369	NS
Final weight (FW), g	340^{b}	362^{a}	364^{a}	370^{a}	3.633	*
Calculation the energy retention						
Energy content in final body fish (cal / g)	5.7361^{d}	5.8774^{c}	6.0729^{b}	6.2287^{a}	0.057	*
Total energy at the end in body fish (E)	1950^{d}	2128^{c}	2211^{b}	2305^{a}	40.14	*
Energy content in initial body fish (cal / g)			5.2533			
Total energy at the start in body fish (E_0)	447	441	436	441	2.00	NS
Energy retained in body fish ($E-E_0$)	1503^{d}	1687^{c}	1775^{b}	1864^{a}	41.04	*
Energy of the feed intake (Cal / g feed)	4.412	4.539	4.635	4.715	-	-
Quantity of feed intake	427.56^{c}	445.20^{ab}	443.10^{b}	451.92^{a}	2.888	*
Total energy of feed intake (EF)	1886^{c}	2021^{b}	2054^{b}	2131^{a}	27.276	*
Energy retention (ER) %	79.69^{c}	83.47^{b}	86.42^{a}	87.47^{a}	0.934	*
Calculation the protein productive value (PPV) %						
Crude protein % in final body fish	60.93^{a}	58.09^{c}	59.04^{b}	55.19^{d}	0.631	*
Total protein at the end in body fish (PR_1)	207.16^{b}	210.29^{ab}	214.91^{a}	204.20^{b}	1.506	*
Crude protein % in initial body fish			65.56			
Total protein at the start in body fish (PR_2)	55.73	55.07	54.41	55.07	0.243	NS
Protein Energy retained in body fish (PR_3) = ($PR_1 – PR_2$)	151.43^{b}	155.22^{ab}	160.50^{a}	149.13^{b}	1.586	*
Crude protein in feed intake (CP %)	29.484	29.85	29.92	29.21	-	-
Total Protein intake (PI), g	126.04^{b}	132.89^{a}	132.58^{a}	132.01^{a}	0.904	*
Protein productive value (PPV) %	120.14^{ab}	116.80^{b}	121.06^{a}	112.97^{c}	1.061	*

SBM: Soybean meal. GML: *Galleria mellonella* larvae. a, b and c: Means in the same row having different superscripts differ significantly (P< 0.05). SEM: Standard error of mean. NS: Not significant. *: Significant at (P< 0.05).

Economical evaluation of different experimental groups

The economic evaluation results of the different experimental groups in Table (8) indicate that incorporating GML into the feed formulation reduced the feed costs from 24.700 LE per kg in the control diet (D1) to 24.340, 23.980, and 23.620 LE per kg for the other diets (D2, D3, and D4, respectively). Furthermore, the net improvement was 6.17, 8.70, and 10.07% for D2, D3, and D4, respectively, compared to the control diet without GML.

Table 8. Economical evaluation of different experimental groups

Item	Tested diets			
	Control Zero GML	**Replacing 5% of SBM by GML**	**Replacing 10% of SBM by GML**	**Replacing 15% of SBM by GML**
	D_1	**D_2**	**D_3**	**D_4**
Costing of kg feed (LE)	24.700	24.340	23.980	23.620
Relative to control diet (%)	100	98.54	97.09	95.63
Feed conversion ratio (FCR)	1.68	1.60	1.58	1.58
Feeding cost (LE) per (Kg weight gain)	41.50	38.94	37.89	37.32
Relative to control (%)	100	93.83	91.30	89.93
Net improving in feeding cost (%)	0	6.17	8.7	10.07

SBM: Soybean meal. GML: *Galleria mellonella* larvae. LE.: Egyptian pound. Diet formulation calculated according to the local prices at year 2023 as presented in (Table 1). Feed cost (L.E) FCR×FI. Cost per Kg diet

DISCUSSION

The current study observed improvements in FW, TBWG, ADG, SGR, and SR in the experimental groups fed diets when 5, 10, and 15% of SBM were replaced with GML. Furthermore, the mortality rate was 0% in these groups (D2, D3, and D4), compared to 6.67% in the control group (D1). Additionally, it was observed that incorporating GML at these levels had no significant impact on the aforementioned parameters. Moreover, FI, FCR, CPI, and PER significantly ($P< 0.05$) increased when SBM quantity used in control diet formulation at 40% was replaced by GML at 5, 10 and 15% in three tested diets of the Nile tilapia fish. These findings are consistent with those reported by **Fayed *et al.* (2023)**, who investigated the use of black soldier fly larvae meal (BSFM) as a substitute for fish meal (FM) in the diets of the Nile tilapia (*Oreochromis niloticus*). In their study, experimental diets were formulated with varying levels of BSFM, specifically 0, 5, 10, 15, 20, 25, and 30%, replacing equivalent amounts of protein in FM. Their results indicated that growth performance, feed conversion ratio, and survival rates improved with the inclusion of BSFM up to a 25% replacement level ($P< 0.001$, 0.017, and 0.001, respectively). Beyond this level, specifically at a 30% replacement, the performance metrics began to decline. Additionally, they observed a dose-dependent increase in feed utilization and survival rates when BSFM replaced up to 25% of FM in the Nile tilapia diets. Similarly, **Aini *et al*. (2018)** found that replacing 50% of FM with a mix of BSFM and *Manihot esculenta* leaf meal improved the Nile tilapia growth.

Furthermore, studies by **Rana *et al*. (2015)** and **Muin *et al*. (2017)** indicated that tilapia fed diets in which 50% of the fish meal (FM) was substituted with BSFM showed growth performance equivalent to the control group. Additionally, research by **Renna *et al*. (2017)** and **Belghit *et al*. (2019)** demonstrated that incorporating BSFM at high levels (40-60% for the Atlantic salmon and 20-40% for the rainbow trout) did not adversely affect growth performance. Moreover, **Li *et al*. (2017)** demonstrated that defatted BSFM could fully substitute FM in the diets of the Jian carp (*Cyprinus carpio*) without adversely affecting growth. The enhanced growth performance observed in this study can be linked to the active ingredients found in BSFM-containing diets, such as essential amino acids, aligning with the findings of **Spranghers *et al*. (2017)** and **Fayed *et al*. (2023)**. Furthermore, the significant levels of omega-3, 6, and 9 fatty acids, as noted by **Liland *et al*. (2017)**, likely played a role in this improvement. Moreover, **Diener *et al*. (2009)** highlighted that BSFM boasts high protein content (60%) and a lipid content ranging from 10-30%, with a more balanced amino acid profile compared to fish meal. On the other hand, **Fayed *et al*. (2023)** observed a decrease in growth performance when 30% of FM was replaced by BSFM, attributing this reduction to the high chitin content in BSFM, which increases with higher inclusion levels. Additionally, **Karlsen *et al*. (2017)** noted that chitin acts as a filler with low digestible energy content and can inhibit growth rates at high inclusion levels. Furthermore, **Alegbeleye *et al*. (2012)** clarified that high chitin concentrations, especially in insect-based diets, can delay amino acid absorption. In addition, **Guerreiro *et al*. (2020)** found that replacing FM with BSFM led to a linear decrease in the growth of the meager at FM substitution levels of 17, 35, and 52%. Additionally, **Dietz and Liebert (2018)** reported a decline in growth performance and feed conversion ratio in the Nile tilapia when 50% BSFM was used to replace soy protein concentrate.

Animal-based protein sources like insect meal (IM) are being explored as potential substitutes for fish meal in aqua-feed. Many studies have focused on utilizing IM in fish farming as a practical feed alternative **(Hua *et al*., 2019; Fisher *et al*., 2020; Li *et al*., 2020)**. Insect meal is recognized as a valuable source of protein, vitamins, and minerals **(Basto *et al*., 2020)** and has been successfully utilized as a natural immunostimulant in fish diets in various studies. For example, **Tippayadara *et al*. (2021)** observed an enhancement in lysozyme and peroxidase activities in the skin mucus of the Nile tilapia after feeding with 4 and 6% black soldier fly larvae meal. Another study was carried out on the yellow catfish fed diets in which 13, 25, 37, and 48% of fish meal protein were replaced by black soldier fly larvae showing an obvious improvement in immune parameters like lysozyme, phagocytic percentage, and phagocytic index **(Xiao *et al*., 2018)**. Additionally, a diet containing BSFM enhanced the innate immune response of the marron (*Cherax cainii*) and increased the the lysozyme activity and total protein **(Foysal**

***et al*., 2019)**. Moreover, incorporating meal worm (*Tenebrio molitor*) as a partial substitute for fish meal in the yellow catfish diets improved humoral immune parameters (lysozyme and IgM) and immune-related genes (MHC II, IL-1, IgM), without negatively affecting fish growth **(Su *et al*., 2017)**. In agreement with previous studies, our study revealed enhancements in immune parameters (lysozyme, total protein, albumin, and globulin) in all fish groups fed diets supplemented with various doses of *Galleria mellonella* larvae. Interestingly, the lowest dose (5%) resulted in the highest immune parameters, supporting the potential of these larvae as an immunomodulatory supplement for the Nile tilapia at a 5% dosage.

Biochemical analyses are crucial for assessing the physiological status of fish and their response to external factors like feeding on new additives or growth promoters **(Abbas *et al*., 2021; Abozaid *et al*., 2024)**. Aspartate and alanine aminotransferases (AST and ALT) are two important liver enzymes typically located within liver cells. Their presence in the bloodstream indicates liver cell damage and disruption in liver function. In our study, AST and ALT levels were insignificantly affected in all groups fed with GML compared to the control, suggesting no impairment in liver function. This finding is consistent with previous research on the European sea bass, recording no changes in AST and ALT levels following dietary insect treatments **(Mastoraki *et al*., 2020)**.

In fish, glucose and cholesterol levels indicate stress and disturbances in metabolic processes **(Sopinka *et al*., 2016)**. Fortunately, in this study, glucose and cholesterol levels showed no significant changes across all groups fed with GML compared to the control group, indicating the safety of using these larvae as a nutritional protein source for fish. However, the present study recorded an increase in serum uric acid levels in *O. niloticus* groups fed higher concentrations of *Galleria mellonella* (15% GML), followed by lower concentrations (10% GML and 5% GML). This increase in uric acid level may indicate an increase in protein and amino acid catabolism for energy purposes since insects have an average protein content of 50-82% **(Henry *et al*., 2015)**. A similar previous study revealed an increase in serum urea and creatinine levels after feeding the gilthead sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) on a protein-rich diet with insect **(Randazzo *et al*., 2023)**.

Feeding the Nile tilapia diets supplemented with GML resulted in significant ($P< 0.05$) increases in fish body composition, including DM, OM, EE, and gross energy content. Meanwhile, the values of moisture, crude protein, and ash contents showed significant ($P< 0.05$) decreases compared to the control diet. These findings are consistent with those reported by **Fayed *et al*. (2023)**, who found that increasing levels of BSFM led to gradual decreases in body lipid levels ($P\leq 0.01$) up to 25% replacement, followed by an increase at 30% BSFM. On the other hand, as the amount of BSFM in the diets increased, the protein content also rose. Additionally, the results indicated that body moisture and ash contents decreased over time with higher levels of BSFM replacement, with the control group and the 25% replacement group ($P\leq 0.01$). Additionally, **Fayed *et al.* (2023)** mentioned that the whole-body proximate composition showed switching from FM to BSFM in the fish diet modified the fish major constituents by increasing moisture contents and decreasing both ash and fish lipid content. Additionally, studies by **St-Hilaire *et al*. (2007)** and **Kroeckel *et al*. (2012)** highlighted significant impacts of dietary BSFM meal on whole-body lipid composition in the rainbow trout and the juvenile turbot. Conversely, **Zhou *et al*. (2018)** and **Abdel-Tawwab *et al*. (2020)** observed no significant changes in kidney index, spleen indices, crude fat, and intraperitoneal fat index in the Jian carp fed low to moderate levels of BSFM. Furthermore, **Belforti *et al*. (2015)** noted that increasing *Tenebrio molitor* larvae meal in the rainbow trout diets significantly increased crude protein content and decreased overall lipid content. In contrast, **Zhou *et al*. (2018)** found no notable variations in crude protein and crude lipid amounts in the whole body, muscles, and hepatopancreas of the Jian carp fed varying doses of BSFM.

Including GML in the Nile tilapia diets at different levels of 5, 10, and 15% (D2, D3, and D4, respectively) significantly (P< 0.05) increased energy retention (ER)% by 4.74, 8.45, and 9.76% compared to the control (D1). Conversely, protein productive value (PPV)% significantly (P< 0.05) decreased in fish groups receiving diets containing 15% GML (D4), while groups fed diets with 5 or 10% GML (D2 or D3, respectively) showed no significant effects (P>0.05) compared to the control diet. Specifically, PPV% improved by 0.77% in the D3 group, but decreased by 2.78 and 5.97% in the D2 and D4 groups, respectively, compared to the control (D1). These findings contrast with those reported by **Abozaid *et al*. (2024)**, who found that including *Saccharomyces cerevisiae* in the Nile tilapia diets at 4, 8, and 12g/ kg significantly (P< 0.05) decreased the ER% values but significantly (P< 0.05) increased the PPV% values.

The economic evaluation of different experimental groups in this study revealed that including *Galleria mellonella* larvae (GML) in feed formulations reduced feed costs from 24.700 LE per kg in the control diet (D1) to 24.340, 23.980, and 23.620 LE per kg for diets D2, D3, and D4, respectively. Furthermore, net improvements of 6.17, 8.70, and 10.07% were recorded for D2, D3, and D4, respectively, compared to the control without GML. These findings align with those of **Fayed *et al*. (2023)**, who showed that increasing levels of BSFM decreased feed costs, improved growth performance and health status, and enhanced the economic feasibility of commercial tilapia diets. Moreover, they reported that increasing BSFM in diets reduced feed costs from 0.70 to 0.63 US$/kg of diet. Similarly, **Hebicha *et al*. (2013)** noted that feed cost, feeding rate, fish output, and sales prices are critical factors affecting profitability and recorded an improvement of FCR with an increase in BSFM in diets replacing FM. Likewise, **Abdel-Tawwab *et al*. (2020)** highlighted that incorporating BSFM (*Hermetia illucens* L.) in fish diets reduced feeding costs by 15.6% per kg. On another note, **Abozaid *et al*. (2024)** reported that including *Saccharomyces cerevisiae* in the Nile tilapia diets at 4, 8 and 12g/ kg significantly improved the net revenue by 6.80, 9.47, and 19.03%, respectively, compared to the control group. Additionally, **Goda *et al*. (2012)** noted that rising feed prices are among the most significant factors limiting the profitability in fish culture. However,

Azevedo *et al*. (2015) explained that the economic efficiency is the product of multiplying productive efficiency by price efficiency. Furthermore, **Abareethan and Amsath (2015)** highlighted that the use of probiotics can lower feed costs, which is crucial for evaluating the feasibility of fish diets.

CONCLUSION

Based on the current findings, it can be concluded that incorporating *Galleria mellonella* larvae as a protein source to partially replace soybean meal at levels of 5, 10, and 15% in fish diets resulted in enhanced growth performance, feed utilization and immunity. This substitution had no effect on cholesterol, glucose, AST, and ALT levels, while increases were detected in the EE and gross energy content of the fish body composition. Additionally, it improved energy retention values and feed conversion ratios, and resulted in net cost savings in feeding. Therefore, replacing up to 15% of the soybean meal in diet formulations of the Nile tilapia with *Galleria mellonella* larvae appears optimal for obtaining ideal growth and production.

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