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Evaluation of Mulberry Silkworm, *Bombyx mori*, Pupae as a Sustainable Replacement for Fishmeal in the Gilthead Seabream (*Sparus aurata*) Diets

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ABSTRACT

This study looks into the impact of partial substitution of the dietary fishmeal (FM) with silkworm (*Bombyx mori*) pupae meal (SP) at 30, 50, and 70% replacement levels, in the juvenile gilthead seabream, *Sparus aurata* diets. Triplicate groups comprising 30 fish (IW=14.50 ± 0.50g) were fed for 58 days on one of four isoprotenic (46%) and isoenergetic (21 MJ/ Kg) diets (SP0, SP30, SP50, or SP70). The amino acid profiling of FM and SP confirmed the presence of all essential amino acids in the pupae, with a high degree of similarity observed between the two protein sources, suggesting functional equivalence for dietary protein replacement. The body weight and specific growth rate of fish, at the end of the experiment, of SP50 and SP70 groups were higher than in SP0 and SP30 fish groups ($P< 0.05$). The fish biochemical composition analysis indicated insignificant variations among all experimental groups. The hematological parameters illustrated that by increasing SP levels in fish diets, hemoglobin, hematocrit, and the red blood cells count recorded values were significantly increased relative to the SP0 group, and SP70 group showed the highest values. Furthermore, the white blood cell count was gradually and significantly increased with increasing the SP substitution level in fish diets. The percentage of neutrophils and lymphocytes was also the highest in SP70 group indicating an enhancement in fish immunity. From the result of our investigation, we can conclude that SP can be a promising alternative for replacement of FM in the gilthead seabream feed up to 70%, and furthermore, reducing fish oil (FO) by 28% with maintaining fish growth, health, and immunity, improving sustainability, and lowering feed costs. Further investigation is advised to look at the long-term effects of this substitution on fish performance.

INTRODUCTION

Due to its well-balanced amino acid content, high palatability and digestibility, fishmeal (FM) is one of the main feed ingredients used to prepare fish feed. These qualities improve the fish's capability to digest and absorb nutrients. The majority of marine fish have high nutritional needs for protein, and because of its nutritional value and pleasant palatability, FM is the best way to provide these needs **(Serra *et al.*, 2024)**.

However, the shortage of FM is rapidly increased as a result of the persistent drop in wild fish captures and the rise in the requirement for high-quality aquaculture feeds. Lack of FM drives up the cost of feeds and accordingly, novel dietary ingredients are required for aquaculture to grow sustainably **(Hussain *et al*., 2024)**. Consequently, research is now focused on replacing FM in aquatic feeds, either completely or partially, with animal or plant proteins **(Refstie *et al*., 2001; Watanabe 2002; Abasubong *et al*., 2021)**. Nonetheless, scientists discovered that while FM can be partially replaced, it cannot be fully replaced in the diet of fish such as the Black Sea turbot **(Yigit *et al*., 2006)** and Atlantic cod **(Olsen *et al*., 2007)**. Aquaculture species have found great success using plant protein in their diets; however, a number of negative issues have been mentioned, such as the existence of several anti-nutritional substances and the imbalanced amino acids composition **(Jannathulla *et al*., 2019; Liu *et al*., 2021)**. The majority of the animal protein origins seem to be more appropriate as dietary substitutes because they are nutritionally more comparable to FM. An integral part of the aquafeed industry is insect meal. One viable substitute protein source among different insect meals is silkworm pupae meal (SP). In the sericulture sector, silkworm pupae remaining after silk fiber reeling are often discarded, but they can be repurposed as a valuable source of animal feed. In aquafeed, SP can be a superior alternative to FM since it is more affordable and easily approachable **(Jeyaprakashsabari & Aanand, 2021)**. SP has a high nutritional value and is widely available. Dry pupae have 24–33% crude lipid and 50–70% crude protein. Silkworm pupae (SP) are a rich source of nutrients, with a well-balanced profile of essential amino acids. Notably, they contain particularly high levels of lysine and methionine **(Ashish, 2022)**.

SP has shown various biological advantages since it is considered as an antioxidant, antimicrobial, and anti-diabetic agent. Furthermore, SP has cardiovascular protective effects, making it suitable for use in pharmaceuticals and health-care products **(Sadat *et al.* 2022)**. It costs far less than FM, although it has a similar nutritional value **(Ji *et al*. 2015)**. Furthermore, **Tariq *et al*. (2025)** reviewed many bioactive components in SP such as polyunsaturated fatty acids, essential amino acids, tocopherols, phospholipids, antioxidants, and antimicrobial peptides. However, its chitin is difficult to digest and is considered as a limiting factor in the usage of SP. Many studies have attempted partly or completely replacing FM with SP in fish diets **(Begun *et al*., 1994; Nandeesha *et al*., 2000; Boscolo *et al*., 2001; Samocha *et al.*, 2004; Salem *et al*., 2006; Xu *et al.*, 2018; Rahimnejad *et al.*, 2019; Sathishkumar *et al.*, 2021; Hodar, 2022)** and shrimp diets **(Rahimnejad *et al*., 2019; Hodar, 2022)** but the studies' results have been controversial. To the authors' knowledge, no previous work examined incorporation of SP in the gilthead seabream diet. In this study, the impacts of SP on the gilthead seabream growth, feed utilization ability, carcass composition, and overall health were assessed to determine the optimal level of SP integration in the diet as a partial replacer for FM and fish oil (FO). These data will increase the usage efficiency of SP and serve as reference information for the creation of an aquaculture feed that is nutritionally adequate for seabream.

MATERIALS AND METHODS

This work was carried out at the Sericulture Department of Plant protection Research Institute, Agriculture Research Center (ARC) and National Institute of Oceanography and Fisheries (NIOF) on the gilthead seabream (*S. aurata*) juveniles.

The domesticated mulberry silkworm, *Bombyx mori* (SP), was collected at the pupal stage after reeling the silk cocoons using reeling machines. The extracted pupae were then dried at 50°C in a specialized oven to remove moisture content. Additionally, pupae from damaged cocoons—those unsuitable for machine reeling—were obtained by manually cutting the cocoons and extracting the pupae (Fig. 1).

Fig. 1. Dried silkworm pupae (SP) (*Bombyx mori*)

Amino acids determination

Extraction procedures

After mixing 100mg of either FM or SP with 5mL of water and 5mL of HCl, the mixture was heated to 100°C for 24 hours before being filtered. Moreover, 1mL of the filtrate was finally dried, reconstituted in 0.1 M HCl, and injected into an HPLC, as described by **Campanella *et al*. (2002)**, **Laurens *et al*. (2012)** and **Igor *et al*. (2013)**.

HPLC analysis

HPLC analysis was performed using an Agilent 1260 Series system equipped with a Diode Array Detector (DAD) and a fluorescence detector. Separation was carried out on an Eclipse Plus C18 column (4.6mm × 250mm, 5µm particle size). The mobile phase consisted of solvent A (sodium phosphate buffer, pH 7.8) and solvent B (acetonitrile: methanol: water, 45:45:10, v/v/v).

The flow rate was maintained at 1.5mL/ min. The following linear gradient program was applied:

Time (Min)	A %	B %
0.00	98	2
0.84	98	2
33.40	43	57
33.50	0	100
39.30	0	100
39.40	98	2
40.00	98	2

A DAD was set to monitor absorbance at 338nm with a bandwidth of 10nm. The column temperature was maintained at 40°C. Fluorescence detection was performed with the excitation/emission wavelengths set at 340/450nm from 0 to 25 minutes and at 266/305nm from 25 to 40 minutes.

Fish rearing

Fish were acclimated for ten days to the rearing system. The initial biochemical composition was determined by sampling ten fish from the original stock population. During that time, fish were fed a commercial diet for sea bream (Aller aqua, Egypt).

A 58-day feeding trial was conducted using juvenile seabream with an approximate start weight of 14.5 ± 0.5g. Fish were indiscriminately allocated into four treatment groups with three replicates per group (thirty fish each). Moreover, fish were maintained under controlled conditions (temperature: 24±0.5°C, salinity: 36.5±1.0, photoperiod: 12h light:12h dark) and fed to apparent satiety three times daily.

Diet preparation

Silkworm Pupae were sourced from the Sericulture Department, Plant protection Research Institute, Agriculture Research Center, dried, and grounded and sieved. FM and SP powder were analyzed for proximate composition and their amino acid profile was identified (Table 1). Four isonitrogenous (Ca. 47% CP) and isolipidic (Ca. 14%) experimental diets were formulated. Fish diets were developed to meet their nutritional needs. According to **Saleh *et al*. (2018)**, dietary components were fully combined and dry-pelleted in a laboratory-scale pellet machine.

FM was progressively replaced with SP at 30, 50, and 70% levels and also, FO was gradually decreased by increasing the SP level in fish diets to keep the total lipid content in all the experimental diets at the level of 14%. After drying, the diets were placed in plastic bags to be used later. Table (2) lists the ingredients and the experimental diets proximate analysis.

Data collection

By the end of the trial, five fish per tank were randomly chosen and pooled for a complete body composition study following a day of fish fasting.

Biochemical proximate analysis

The **AOAC (2012)** protocol was followed in the biochemical analysis of fish and diets. Proximate chemical tests of the moisture, crude protein, ether extract, crude fiber, and ash contents were performed on fish samples and tested diets. The samples were dried using an oven (100°C) until their weight remained constant in order to estimate the moisture content. The Kjeldahl method (Velp Scientifica, Italy) was used to assess the crude protein content, and a solvent mixture of methanol and chloroform was used to extract the lipid content. Ash was measured by burning the sample for four hours at 600°C in a muffle furnace until the weight remained constant.

Table 1. Proximate composition (%) and amino acid profile (g/100 g protein) of fishmeal (FM) and silkworm pupae meal (SP) (*B. mori*)

Proximate composition (%)	**Experimental ingredients**	
	FM	**SP**
Total protein	68	58.9
Total lipid	12.5	19.1
Ash	12	8
Essential Amino Acids		
Arginine	6.01	5.32
Histidine	2.95	3.18
Isoleucine	4.20	3.79
Leucine	7.41	5.66
Lysine	6.99	4.78
Methionine	3.00	2.62
Phenylalanine	4.21	3.84
Threonine	4.42	5.12
Valine	5.04	3.9
Non-Essential Amino Acids		
Alanine	5.09	4.62
Aspartic Acid	7.11	9.94
Glutamic Acid	10.41	14.7
Glycine	5.50	4.8
Serine	4.04	4.56
Tyrosine	3.51	4.66
Cystine	0.99	1.42
Proline	4.00	2.94

Table 2. Proximate and biochemical composition (% DM) of the tested diets fed to sea bream (*S. aurata*) for 58 days

Ingredients	**Diets (g/Kg)**			
	SP0	**SP1**	**SP2**	**SP3**
Fish meal1 (FM)	480	336	240	144
Soybean meal[2]	200	200	200	200
Silk worm pupae meal[3] (SP)	0	150	250	350
Corn gluten[4]	70	76	82	87
Wheat flour	133	127	126	122
Shrimp meal	7	7	7	7
Fish oil[5] (FO)	72	66	63	52
Lecithin	5	5	5	5
Vitamins and Minerals mix[6]	30	30	30	30
vitamin C	3	3	3	3
Proximate analyses (% DM)				
Dry matter (DM)	91.09	90.18	91.02	90.84
Crude protein (CP)	46.30	46.05	46.14	46.31
Total Lipids (L)	13.64	14.04	14.12	14.00
Ash	9.82	10.48	10.60	10.71
Fiber	1.80	2.45	2.60	2.47
Nitrogen Free Extract (NFE)[7]	27.14	26.08	26.38	26.51
Gross energy (MJ/Kg)[8]	21.28	21.10	21.11	21.00

[1] FM Lab. made & [2] 44% CP & [3] Agricultural research center & [4] 63% CP & [5]FO, Univela, Morocco & [6] Vitamins and minerals premix (mg kg^{-1}): p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca pantothenate (56.88); pyridoxine HCl (11.38); riboflavin (7.58);thiamine HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamine (0.08); menadione (3.80), vitamin A palmitate (17.85); a-tocopherol (18.96); calciferol (1.14). $K2PO_4$ (2.01); Ca3 $(PO4)_2$ (2.736); Mg SO_47H_2O (3.058); NaH2PO₄ → $NaH2PO_4$ $2H_2O$ (0.795).

[7] NFE, calculated by difference & [8] Gross energy was calculated based of 23.6, 39.4 and 17.2 MJ/Kg for protein, lipid, and carbohydrate, respectively **(NRC, 2011)**

Growth response and feed performance

At the end of the experiment, the fish in each tank were carefully weighed and counted to determine the growth parameters. From each tank, five fish were randomly chosen, and their total length and weight were measured to calculate the condition factor (K).

Growth performance and feed efficiency were evaluated as follows:

Growth performance and feed utilization indices were calculated according to the methods described by **NRC (2011)**. Weight gain (WG, g) was determined as the difference between final and initial body weight: WG = final weight (g) – initial weight (g). Specific growth rate (SGR, % day^{-1}) was calculated using the formula: SGR = [ln (final weight) – ln (initial weight)] × 100 / number of experimental days. Feed conversion ratio (FCR) was calculated as feed intake divided by weight gain. Protein efficiency ratio (PER) was calculated as: PER = protein intake / weight gain. The condition factor (K) was determined using the formula: K = 100 × TW / L^3, where TW is the total body weight (g), and L is the total body length (cm).

All experimental procedures involving fish were approved by the Research Committee at the National Institute of Oceanography and Fisheries (NIOF), Egypt. The study was conducted in accordance with the Guide for the Use of Laboratory Animals (European Communities Council Directive 2010/63/EU).

Hematological analyses

For the purpose of drawing blood, five fish were taken randomly from each tank (15 fish per treatment). For complete blood count (CBC) analysis, a heparinized syringe was used to draw blood, which was then kept at 4°C for 30 minutes before analysis. A Fully Automatic Blood Cell Counter (model PCE-210 N, Erma, Inc. India) was used to determine the CBC of each blood sample. The following parameters were measured: mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH, pg), MCH concentration (MCHC, g/dl), white blood cell count (WBC, 10^3/µl), hemoglobin concentration (Hb, g/dl), haematocrit value (Hct, %), and red blood cell count (RBC, 10^6/µl). Additionally, the differentiation of WBCs was evaluated.

Statistical analysis

For every measured parameter, the mean value and standard error (mean ± SE) were first determined. ANOVA, or one-way analysis of variance, was applied to the results. The SPSS software was used to analyze the data **(SPSS, 2016)**. The Duncan multiple range test was applied to compare between the mean differences at the $P< 0.05$ level.

RESULTS

Amino acids (AAs) analysis for FM and SP indicated that SP contains all essential amino acids (EAAs) and FM slightly outperforms SP in most EAAs, especially lysine (6.99 vs. 4.78) and leucine (7.41 vs. 5.66). In contrast, SP has higher threonine (5.12) and histidine (3.18). Non-essential amino acids (NEAA) analysis showed that SP contains higher aspartic acid (9.94 vs. 7.11), glutamic acid (14.7 vs. 10.41) and cystine (1.42 vs. 0.99).

The fish growth performance and the feed utility results are summarized in Table (3). Obtained results show significant variations ($P< 0.05$) in WG values. Fish fed SP50 and SP70 diets showed significant elevation in their recorded values (15.65 & 15.71) relative to the control group (SP0, 11.77) and insignificant elevation relative to SP30 group (13.46).

Table 3. Growth rates and feed utilization effectiveness of seabream *(S. aurata)* fed diets the experimental diets for 58 days. Values are expressed as means ± SE

Parameters	Dietary fish group				
	SP0	**SP1**	**SP2**	**SP3**	***P*value**
Initial weight, IW (g/fish)	14.75±0.06	14.43±0.23	14.45±0.13	14.51±0.17	0.003
Final weight, FW (g/ fish)	26.52±1.01^{b}	27.89±1.70^{b}	30.07±0.94^{a}	30.22±0.75^{a}	0.004
Weight gain, WG	11.77±0.95^{b}	13.46±1.90^{ab}	15.65±1.10^{a}	15.72±0.76^{a}	0.012
Specific growth rate, SGR (%/ day)	1.01±0.06^{b}	1.13±0.13^{b}	1.27±0.07^{a}	1.26±0.05^{a}	0.041
Feed intake, FI (g /fish)	25.64±0.30	28.76±0.13	27.10±0.17	25.67±0.44	0.544
Feed conversion ratio, FCR	2.18±0.07^{a}	2.05±0.16^{a}	1.79±0.15^{b}	1.63±0.78^{b}	0.03
Protein efficiency ratio, PER	1.00±0.47^{b}	1.05±0.13^{b}	1.21±0.04^{a}	1.32±0.06^{a}	0.020
Condition factor (K)	1.35±0.09^{c}	1.51±0.10^{b}	1.54±0.05^{b}	1.64±0.02^{a}	0.01

Values in the same row with different superscripts are significantly different ($P< 0.05$).

Regarding the SGR values, both SP50 and SP70 groups showed significantly higher values (1.26 & 1.27 respectively) comparing with SP0 and SP30 groups (1.01 & 1.13 respectively). Concerning the feed utilization efficiency, FI values indicated insignificant variation among all dietary groups ($P> 0.5$) indicating no negative effects on fish appetite among all dietary groups. The best FCR value was observed in SP70 (1.63) followed by SP50 group (1.79). Lower FCR in SP50 and SP70 suggests that they utilized feed more proficiently and suggesting that SP70 is the most efficient diet. SP70 and SP50 groups showed higher PER values (1.32 & 1.21, respectively) relative to SP30 and SP0 groups (1.05 & 1.09, respectively). K value was significantly elevated in the all tested fish groups comparing with SP0 group (1.35) and the highest value was recorded in SP70 group (1.64). SP50 and SP70 had higher PER relative to SP0 and SP30, indicating better protein utilization. Overall, SP50 and SP70 appear to be the best-performing dietary groups for growth and feed efficiency.

The outcomes of the fish biochemical analyses, at the terminal of the experiment, are summarized in Table (4). The results indicated insignificant variation ($P>0.05$) among all experimental groups in regard of moisture, protein, fat and ash contents.

Table 4. Proximate chemical composition (% on wet weight basis) of the whole-body of seabream, *S. aurata*, fed diets the experimental diets for 58 days. Values are expressed as means ± SE (n=3)

Parameter	**Dietary SA groups**				***P*-value**
	SA0	**SA1**	**SA2**	**SA3**	
Moisture	67.10±0.10	67.26±1.13	67.80±0.77	66.90±1.27	0.885
Crude Protein	15.49±0.39	16.67±0.47	14.95±0.29	15.85±0.12	0.144
Ether extract	10.17±0.55	9.66±0.58	8.80±0.46	9.64±0.17	0.294
Ash	5.16±0.42	5.58±0.23	6.13±0.37	5.33±0.32	0.544

Values in the same the row are insignificantly different ($P> 0.05$).

Generally, RBCs, Hb, and Hct recorded values (Table 5) were gradually increased with increasing the SP level in fish diets. However, MCH showed no significant alterations ($P> 0.05$) among the experimental groups, while MCHC exhibited slight variations, with SP70 achieving the highest value (19.05 mg/dL). WBC count is obviously increased in treated fish groups compared with SP0 group, peaking in SP70 (77.48×10^3/mL, $P< 0.05$). Neutrophil percentages also displayed a significant rise ($P< 0.05$), while lymphocyte levels insignificantly increased in treated groups in comparison with SP free group.

Platelet counts varied, but there was no statistically significant alteration ($P> 0.05$), and eosinophil and basophil levels did not differ significantly either.

Table 5. Stress parameters of seabream (*S. aurata)* fed on diets with graded levels of silkworm pupae (SP) for 58 days. Values are expressed as means ± SE (n = 3)

Parameter	CTRL	SP30	SP50	SP70	*P* value
RBC (×10⁶/L)	1.95 ± 0.05^{b}	2.04 ± 0.01^{ab}	2.29 ± 0.26^{ab}	2.47 ± 0.07^{a}	0.044
Hemoglobin (Hb, g/dL)	5.40 ± 0.12^{b}	6.00 ± 0.06^{ab}	6.60 ± 0.86^{a}	6.87 ± 0.41^{a}	0.017
Hematocrit (Hct, %)	28.40 ± 0.75^{c}	32.10 ± 0.46^{b}	36.87 ± 5.11^{ab}	39.60 ± 1.59^{a}	0.035
MCV (FL)	157.40 ± 3.18^{b}	160.43 ± 1.59^{ab}	160.10 ± 4.65^{ab}	166.05 ± 0.2^{a}	0.043
MCH (pg)	29.40 ±0.12	28.73 ±3.22	28.70 ±0.17	27.80 ±0.17	0.503
MCHC (mg/dl)	18.70 ± 0.46^{ab}	17.37 ± 0.67^{b}	17.93 ± 0.28^{ab}	19.05 ± 0.09^{a}	0.041
Platelets	303.00±36.95	336.00±21.73	342.40 ±77.62	378.50±19.92	0.407
WBC (×10³/mL)	34.88 ± 11.37^{d}	56.00 ± 3.61^{c}	63.79 ± 4.45^{b}	77.48 ± 8.75^{a}	0.003
Neutrocytes (%)	41.10 ± 2.25^{b}	42.10 ± 7.92^{ab}	44.27 ± 2.88^{a}	46.25 ± 2.45^{a}	0.035
Lymphocytes (%)	34.10 ±6.29	37.70 ±5.23	43.07 ±4.24	43.30 ±2.14	0.158
Eosinophils (%)	1.05±0.32	1.63±0.12	0.83±0.26	0.85±0.20	0.133
Basophils (%)	17.95±0.8	17.67±2.58	12.40±2.86	14.15±0.14	0.198

Values in the same the row with different superscripts are significantly different (*P < 0.05)*
RBC: The red bloods cell count, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: MCH concentration, WBC: white blood cells count.

DISCUSSION

Due to the nutritional significance of insect meals and the unsustainable production of FM, more researches have been conducted to evaluate the utility of insect meals as FM replacers in the gilthead seabeam diets **(Henry *et al*., 2022; Mastoraki *et al*., 2022).** The impacts of substituting SP meal for FM on the gilthead sea bream growth, feed consumption, carcass analysis, and overall health have not, as far as the authors are aware, been studied before. However, using SP meal as a replacer to FM in other aquaculture fish species diets was investigated **(Ji *et al*., 2015; Zhou *et al*., 2017; Xu *et al*., 2018; Rahimnejad *et al*., 2019; Sathishkumar *et al*., 2021; Hodar, 2022; Bagheri *et al*., 2025)**. According to the study's findings, SP50 and SP70 had significantly higher SGR relative to SP0 and SP30 meaning they grew faster. SP50 had slightly lower efficiency but similar growth to SP70, meaning both diets are beneficial. These results proved that the gilthead seabream's diets can contain up to 70% SP in place of FM protein without having an unfavorable effect on feed consumption or growth performance. Regarding the efficiency of feed utilization, SP30 had higher FI, but its growth was lower than SP50 and SP70, suggesting lower feed efficiency. SP70 had a lower FI than SP50 but still reached a similar final weight, showing better feed utilization. This enhancement in feed efficiency can be attributed to the growth hormone genes (GH) as well as appetite genes (Ghrelin) that were up-regulated in the fish groups fed on SP and also, a significant increase in protein metabolism (TOR) gene expressions, which showed favorable effects of SP supplementation in fish diets **(Bagheri *et al*., 2025)**. SP70 was the most efficient diet, as it provided the best growth with lower feed intake. SP50 was also effective, but its feed intake was higher, which may increase the feeding costs. The good-quality protein content and advantageous amino acids profile of SP are responsible for the same performance at SP50 and SP70 **(Kwon *et al*., 2012)**.

The impacts of substituting graded levels of fermented SP for FM in the diets of the largemouth bass, *Micropterus salmoides*, on growth and feed utilization were examined by **Zahang *et al*. (2022)**. The findings illustrated that the fish at the 30% substitution level had significantly higher WG rates, SGR, feed efficiency rates, and protein efficiency rates than the fish in the other groups. Fish in this group, however, consumed far less feed than those in other groups. Moreover, **Bagheri *et al*. (2025)** demonstrated that permutation of FM protein up to 15% with SP into the diet of fingerling Beluga sturgeon (*Huso huso*) is promising on growth, health and immunity. They concluded that SP could be successfully introduced into the fish feed. In juvenile mirror carp, *Cyprinus carpio*, enzymatic hydrolysates of defatted SP replaced up to half of FM without negative impacts on growth and improved non-specific immunity **(Ji *et al*., 2015)**. The positive effects on fish growth, when SP was added to fish diets, may be attributed to that SP is high in protein (~59%) and contains beneficial lipids (~19 %), making it a good substitute for both FM and FO. It provides essential fatty acids (ω-3 &

ω-6), improves growth, and enhances immune function. Furthermore, SP contains a high ratio of eicosapentaenoic acid (0.3%). Moreover, they contain vital nutrients, such as riboflavin (vitamin B2), quercetin diglucoside, and antioxidants **(Kwon *et al*., 2012)** and all these bioactive materials improve growth, and enhance immune function.

The present results indicated insignificant variations in the carcass biochemical composition among all dietary groups. The current findings are consistent with those of **Salem *et al*. (2008)**, who reported that the proximate composition of the Nile tilapia (*Oreochromis niloticus*) was not significantly affected by the inclusion of silkworm pupae (SP) as a fishmeal (FM) replacement in both experimental and control diet groups.

The blood analysis results indicated significant physiological improvements across the different treatments, with SP70 showing the most pronounced positive effects expressed by a significant elevation in the RBCs count, Hb and Hct levels, relative to the other experimental groups, suggesting an enhanced oxygen-carrying capacity. Moreover, MCV increased significantly, indicating a trend toward larger red blood cells in SP70. WBC count significantly increased with treatment, peaking in SP70, indicating a potential enhancement in immune response. Moreover, platelet counts showed no statistically significant difference, suggesting that the treatments did not adversely affect the coagulation function. No significant differences were observed for eosinophil and basophil levels, implying no major changes in the inflammatory responses. In general, the blood parameters indicated an enhancement in the fish health and immunity when SP was added to fish diets. Higher lymphocyte levels generally enhance adaptive immunity, improving antibody production and long-term disease resistance **(Mokhtar *et al*. 2023)**. The present investigation suggests that fish given SP50 and SP70 diets had a little ($P>0.05$) increase in lymphocytes that may be attributed to the existence of polyunsaturated fatty acids, necessary amino acids (arginine, glutamine), and immunostimulants that boost T-cell and B-cell function.

Herein, the increase in neutrophils in treated groups compared to control values indicating normal immune response as the neutrophils are the first responders in fighting pathogens. The increase in neutrophils may be due to that SP is rich in antimicrobial peptides, chitin, and essential fatty acids that can stimulate innate immune responses and can enhance disease resistance. The decrease in basophils in fish fed on SP could indicate several physiological or immune-related responses. Basophils are a type of white blood cell (WBC) involved in immune responses, particularly allergic reactions and inflammation. Possible reasons for basophil decrease in fish fed on SP is the anti-inflammatory effect of SP, where SP contains bioactive substances that may have anti-inflammatory effects, including PUFAs, antioxidants, and antimicrobial peptides. This could reduce immune activation, leading to a lower basophil count. In conclusion, the present inclusion of SP in fish diets can boost immunity (higher neutrophils & lymphocytes).

The effects of adding SP to fish diets on hematological markers have, regrettably, not been extensively studied in aquaculture fish. In gift tilapia fingerlings (*Oreochromis niloticus*), RBCs, WBCs, Hct, MCV, and Hb concentrations were significantly improved in the SP40 group relative to SP0 group **(Hussain *et al*. 2025)**. In contrast, **Shakoori *et al*. (2015)** investigated the effects of substituting FM with graded percents of SP (5, 10, and 15% replacement levels) on hematological parameters of the rainbow trout (*O. mykiss*). Their results indicated that the values of RBCs and Hb were depleted significantly with elevating the SP percent in fish diets and fish fed a diet containing 15% SP showed the least levels of RBCs and Hb. Furthermore, regarding Hct levels, they recorded no discernible variations existing between experimental treatments. In contrast, the higher the SP percentage in the diet, the higher the MCH, MCV, and WBC values. Additionally, there were no discernible fluctuation among the experimental treatments in the MCHC levels. They conclude that incorporation of SP in the rainbow trout diet can affect their health status. This contradiction in the effects of SP incorporation, in the experimental diets, on fish hematological parameters between the present results and **Shkoori *et al*. (2015)** results may be attributed to the differences in the fish physiology, experimental conditions and diet formulation.

CONCLUSION

Better growth performance and feed conversion competence were recorded when SP was substituting FM up to 70% and FO to 28% without any significant alterations in carcass biochemical composition. The data suggest that SP70 is the most effective treatment in improving RBC, Hb, Hct, and WBC levels indicating enhanced oxygen transport and immune response. This investigation suggests that silkworm pupae are advised as FM replacer in the gilthead seabream diets, because of the insect's high nutritional value and also a cheap alternative as it is a secondary product besides the production of high -value natural silk filaments in addition to that it is an environmentally friendly insect and supports sustainability.

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