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Growth Performance and Haematological Parameters of the Ornamental Fish, *Maylandia estherae*, Fed Varying Inclusion of Silkworm Pupae Meal

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Abstract: The effect of partial replacement of fish meal with silkworm pupae (SWP) meal (*Bombyx mori*) in ornamental fish *Maylandia estherae* was undertaken with five dried diets formulated at 35% crude protein were fed to fingerlings of an average initial weight of 48.83±0.44 g at 0 (control diet), 20%, 40%, 60% and 80% inclusion levels of silk worm pupae meal for 30 days. Fish fed supplemented with silkworm pupae meal showed significantly improved growth performance and feed utilization over the control diet. The weight gain, feeding rate, growth rate, cross conversion efficiency, specific growth rate and feed conversion ratio were measured. Results indicated that, growth performance and feed utilization values were significantly ($P<0.05$) higher in fish fed with diets containing 60% silkworm pupae meal, whereas fish fed diets containing 80% silkworm pupae meal had lower performance. Also, there was an importance in blood parameters (WBCs, RBCs and Hb) and physiological parameters (O_2 carrying capacity and rate of O_2 consumption) in fish fed with SWP meal compared with control diet. The obtained results recommended that, silk worm pupae meal can be replaced in the fish meal up to 60% substitution level of *Maylandia estherae.*

Key words: *Bombyx mori* · *Maylandia estherae* · Growth Performance · Blood Parameters

INTRODUCTION

Ornamental fishes are attractive colourful fishes with various characteristics which are kept as pets in an enclosed space of an aquarium or in a garden pond. These fishes can also be known as 'aquarium fishes' due to its common artificial habitat of aquarium tanks. In recent days the aquaria has been entered in to the houses, schools, tourist places, laboratories, offices, markets, colonies for amusement, education and are providing relaxation to the mind and reduce the depression. Ornamental fishes are offered not only entertainment for hobbyist but also a pleasing profession for many due to escalating to global industry [1]. The production and trade of ornamental fishes are a profitable alternative in the aquaculture sector. Fresh water and marine species have been used successfully in the aquarium fish trade [2].

From nutritional point of view, the nature or origin of the ingredients generally does not matter. Fundamentally the animal is only looking at meeting it is requirement for specific nutrients. The use of a combination of ingredients is therefore necessary to combine ingredients to obtain a mixture that fulfils all the requirements of the animal. One advantage of ornamental fish over farmed fish is the requirement of low amount of food [3]. The Fish preferably utilize proteins for energy purpose, although conventional energy sources such as carbohydrate and lipids may also to some extent to satisfy their needs [4]. The performance of fish meal as a feed ingredient is quite enormous. It contains high level of protein and appreciable quantity of fats and minerals. In some developing countries of the world, fish meal is very scarce and expensive. Besides, availability and high cost, the quality of fish meal is quite uncertain due to the use of different parts of fish and different processing technologies in its production. In addition, this often contaminated with other ingredient such as sand, saw dust and fish bones and use of chemicals for preservation often caused toxicity [5]. Traditionally, fish meal is the deficient in one or more essential amino acids and preferred dietary protein source for many farmed fish species and is appreciated for its amino acid balance, vitamin content, palatability and un-identified growth factors. However, increasing cost of fish meal has created an inadequacy of its use as a protein source for fish diets. Therefore, plant proteins and animal proteins are the good alternatives source for fish meal in fish diets.

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Knowledge on haematological characteristics is an essential tool used as an effective and sensitive index for monitoring physiological and pathological changes in fishes [6]. The assay of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide consistent information on metabolic disorders, deficiencies and chronic stress status they are present in a clinical setting [7].

However, few works has been done on utilization of silkworm pupae meal as feed ingredient. Solomon and Yusuf [8] analyzed the silkworm pupae meal for consumption purpose. Silkworm pupae meal has high protein nutritional value. The alternative raw material is investigated to reduce feed cost and perform the high quality seed production. Silkworm pupae can be served as an additive in the fish meal. It is a by-product of silk thread factory. It has high protein and lipid value as follows (49.4%-60.9% CP) and lipid (14.2%-30.3% C Fat). Amino acid profile closed to fish meal (55% CP). The fatty acid compositions of lipids of *Bombyx mori* L. are oleic, palmitic, palmitoleic, stearic, linoleic (24.6% of total lipid), lauric, myristic, linolenic(14% of total lipid) and arachidic acids that found in saponifiable fraction of neutral lipids fraction. The unsaturated fatty acids usually constitute in one-third of the total acids. Cholesterol, β-sitosterol and a trace of campesterol are found to be present in an oily fraction for sterols. The information on mineral and vitamin content of silkworm pupa are limited [9, 10]. This study investigates on the growth performance and haematological changes of the red zebra (*M. estherae*) using *B. mori* pupae meal dietary supplementation.

MATERIAL AND METHODS

Experimental Animal: The experimental animal, *Maylandia estherae* were collected from the local fish farm in Thoothukudi, Tamil Nadu and immediately transported to the Lab in a separate tank with the same water. These fishes were acclimatized to the lab condition for a month. During acclimatization the animals were fed with dried pellets. The water of rearing tank was changed periodically. After that they were transferred to the experimental tanks which are grouped in to two tanks as one set.

Experimental Diet: For the experimental supplementary feed, silkworm pupae meal was added along with chosen ingredients. The pupae were collected from Government's sericulture farm at Nannagaram near Tenkasi, Tirunelveli District of Tamil Nadu, India. The waste outer layer was removed from the collected pupae. Then pupae were dried in the sunlight for 10 days. After complete drying they were ground and made it to a powder. Then the powder was mixed with chosen ingredients at different levels. The experimental diets were prepared by according to Hardy [11] method. Diets were formulated by including SWP meal at 0, 20%, 40%, 60% and 80% and designated diets as D1, D2, D3, D4 and D5 respectively.

Growth parameters such as weight gain, Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Gross Conversion Efficiency (GCE), Feeding Rate (FR), Growth Rate, Weight of fish and Feed intake were calculated as follows:

Weight gain = Final body weight – Initial body weight

$$FCR = \frac{Totaldryweightoffoodconsumed}{Totalwetweightgain(g)}$$

$$SGR = \frac{Finalwetweight - Initialwetweight}{No.ofdays(t)} \times 100$$

$$GCE = \frac{Growth\,\mathrm{int}\,ermsofdryweightgain(production)}{Totaldryweightoffooconsumed}$$

$$FR = \frac{Feedconsumed}{Initialweightoffish \times NOofdays}$$

All data were expressed as the mean ± SE. Growth and haemotogical parameters data of groups were analysed for significant differences by student "t" test (P<0.05,0.01) and using one-way ANOVA (P<0.05).

RESULTS AND DISCUSSION

The proximate composition of silkworm pupae meal is shown in Table 1. The crude protein, fat, moisture, glycogen, chitin and ashes were found as 48.4%, 32.51%, 7.18%, 4.65% and 4.47%, 4.59% respectively.

Table 1: Proximate composition of *Bombyx mori* pupae meal

Components	Amount (%)
Protein	48.4
Fat	32.51
Moisture	7.18
Glycogen	4.65
Chitin	4.47
Ash	4.59

Table 2: Growth performance and food utilization efficiency of *Maylandia estherae* fed the experimental diets.

Rearing periods (Days)	D1	D2	D3	D4	D5
		Wet weight of fish (g)			
0	48.83± 0.44	48.83± 0.44	48.83± 0.44	48.83± 0.44	48.83± 0.44
15	51.44± 0.33	53.84 ± 0.04.	55.71 ± 0.49	59.45 ± 0.47	50.6 ± 0.47
30	53.26± 0.41	55.11 ± 0.49	57.26 ± 0.28	62.00 ± 0.94	52.36 ± 0.36
		Weight gain (g)			
15	2.60± 0.76	5.01± 0.48	6.87 ±0.72	10.61± 0.23	1.76 ± 0.6
30	4.43 ± 0.51	6.28± 0.58	8.42± 0.25	13.39 ± 0.49	3.53 ±0..23
		Feed intake (g dry matter)			
15	13.39 ±0.021	13.45± 0.032	13.50± 0.045	13.59 ± 0.045	10.90 ± 0.087
30	14.10 0.010	14. 54 ±0.029	14.60 ± 0.054	14.71 ± 0.058	11.82 ±0.065
		Feeding rate(mg g-1 live fish day-1)			
15	16.13 ± 5.98	17.77 ±1.63	18.12± 2.20	19.16 ± 2.38	15.56 ±2.43
30	20.17± 2.40	22.91± 2.10	24.55 ±2.90	25.11± 4.01	18.98± 4.07
		Growth rate			
15	3.55± 0.028	5.55 ± 0.67	7.21± ±1.12	7.90 ± 1.34	2.89 ±1.79
30	4.55 ± 0.69	6.87 ±1.87	8.90 ± 1.55	10.65 ± 1.65	4.21 ± 2.88
		Gross conversion efficiency (%)			
15	20.78± 2.17	25.65 ± 3.03	30.65 ± 3.43	40.38 ± 2.78	19.33 ± 9.85
30	26.97 ± 3.19	32.65 ± 2.56	44.60 ± 2.83	60.64 ± 8.87	24.61± 10.81
		Specific growth rate(% day-1)			
15	17.37± 5.07	33.44± 3.25	45.84 ±4.85	70.77 ± 1.54	10. ±1.18
30	14.76 ±1.72	20.94 ±1.96	28.08 ±0.86	44.64 ± 1.63	11.77± 0.78
		Feed conversion ratio			
15	5.87± 0.65	4.92 ± 0.62	3.01 ± 0.78	2.28 ±0.94	6.87± 0.307
30	6.03 ± 1.76	5.90 ± 2.87	5.01 ± 3.77	2.76 ± 0.13	5.59± 0.363

Each value is the mean (± SD) of three estimations.

The present experimentation the efficiency silkworm pupae meal 20%, 40%, 60% and 80% as fish fed had been evaluated using ornamental fish *Maylandia estherae* as the experimental species. During the feeding trial the fishes accepted different levels of experimental diets (0, 20%, 40%, 60% and 80%). The results have shown that final wet weight of fish, weight gain, specific growth rate and feed conversion ratio of *M. estherae* were affected significantly ($P<0.01$) and feed intake, feeding rate, growth rate, cross conversion efficiency were affected significantly ($P<0.05$). The highest significant values of wet weight of fish, weight gain, feed intake, feeding rate, growth rate and cross conversion efficiency were obtained with the fish maintained at 60% of experimental diets. They were found to be (62.00 ± 0.94, 13.39 ± 0.49, 14.71 ± 0.058, 25.11± 4.01, 10.65 ± 1.65, 60.64 ± 8.87) respectively. Specific growth rate (SGR) improved with increasing the inclusion rate of silkworm pupae meal in the diet (Table 2). The significant improvements ($P<0.01$) in SGR were achieved in 60% of experimental diets. The least FCR value was recorded in 60% of diets which was (2.76 ± 0.13). However, the lowest values were recorded in fish maintained with 80% of diets. They were found to be (52.36 ± 0.36, 3.53 ±0.23, 11.82 ±0.065, 18.98± 4.07, 4.21 ± 2.88, 24.61± 10.81) respectively.

Results of the present work indicate the growth performance of *M. estherae* significantly increased when fed with different levels of silkworm pupae meal incorporated diet. The final body weight, FCR, SGR, FR and GCE of *M. estherae* improved significantly with increasing silkworm pupae meal fed diet up to 60% (D3). Increasing silkworm pupae meal level beyond 60% had no significant effects on growth. These results are in agreement with those obtained by Nandeesh *et al.* [12] who found that using of silkworm pupae meal in the ratio of 30% supplementation has increased the growth rate of common carp. Similarly Begun *et al.* [13] found that significantly better specific growth rate, FCR and PER in rohu fed with 50% silkworm pupae diet compared with fishmeal diet. Similarly Hossain *et al*. [14] stated the,

Table 3. Haematological and physiological parameters of *Maylandia estherae* fed the experimental diets

Rearing period (Days)	D1	D2	D3	D4	D5
			RBC ($\times 10^{-6}$ mm^{-3})		
0	0.32 ± 0.020	0.32 ± 0.020	0.32 ± 0.020	0.32 ± 0.020	0.32 ± 0.020
15	0.94 ±0.032	1.04 ± 0.024	1.10± 0.012	1.29 ± 8.0016	1.24 ± 0.032
30	1.25 ± 8.0016	1.29 ± 08.0016	1.34 ±0 16	1.39 ± 0.024	1.32 ±0.020
			WBC ($\times 10^{-3}$ mm^{-3})		
0	11.33 ± 1.24	11.33 ± 1.24	11.33 ± 1.24	11.33 ± 1.24	11.33 ± 1.24
15	21 ±1.63	25± 0.816	27 ± 0.816	30 ± 0.816	28 ± 0.816
30	24 ± 1.63	36 ±0.816	31 ± 1.25	36± 0.816	33.33± 1.24
			HB (g %)		
0	2.63 ± 0.012	2.63 ± 0.012	2.63 ± 0.012	2.63 ± 0.012	2.63 ± 0.012
15	2.94 ± 0.018	3.03 ±0.047	3.6 ±0.216	3.8 ± 0.047	3.5 ± 0
30	3.5 ± 0.081	3.6 ± 0.08	3.8± 0.047	4 ± 0.81	3.6± 0.081
			O_2 carrying capacity (ml O_2 g^{-1} Hb)		
0	0.62 ± 0.88	0.62 ± 0.88	0.62 ± 0.88	0.62 ± 0.88	0.62 ± 0.88
15	2.10 ± 0.012	2.13 ± 0.012	2.21 ± 0.026	2.44 ± 0.029	2.41 ± 09.004
30	3.18± 0.016	4.07 ± 0.020	4.15± 04.007	4.21 ±0.012	4.05 ± 0.037
			Rate of o_2 consumption (mgo$_2$ $g^{-1}hr^{-1}$)		
0	19.7± 4.37	19.7± 4.37	19.7± 4.37	19.7± 4.37	19.7± 4.37
15	13.3 ± 0.26	12.9 ± 0.08	12.5 ± 0.40	10.46 ± 0.36	10.83 ± 0.17
30	9.6 ± 0.12	7.5 ± 0.08	7.1 ± 0.08	6.6± 0.21	7.1 ± 0.08

Each value is the mean (± SD) of three estimations.

Silkworm pupae meal could be used as a substitute for fish meal at up to 75% of protein in Asian Stinging catfish (Heteropneustes fossilis) diets without adverse effect on growth. Similarly, the comparison between silkworm pupae meal and plant leaf meals (Alfalfa and mulberry) FCE, nutrient digestibility and nutrient retention were better for diets based on silkworm meal than for diets based on plant leaf meals [15]. Ravi Shankar and Keshavanath [16] reported that, the shrimp of *Macrobranchium rosenbergi* utilized pellet feeds containing silkworm pupae and shrimp waste more efficiency and better specific growth rate than diets containing fish meal alone. According to Venkatesh *et al.* [17] the diet containing silkworm pupae enhances the growth of cat fish when compared to meat meal and groundnut oilcake. And also Olaniyi and Babasanmi [18] suggested that, the 100% silkworm pupae meal (*Anaphe infracta*) diet enhances better growth performance in African cat fish.

Table 3 shows haematological parameters and physiological of the fish fed at varied levels of SWP meal. The earned results indicated that the fish fed with SWP meal supplemented diets had a significantly higher ($P<0.05$) white blood cells (WBCs), red blood cells (RBCs) and haemoglobin (Hg) and also physiological parameters like O_2 carrying capacity and rate of O_2 consumption compared to those fed with the control diet.

Blood parameters are considered patho physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicant [19]. Erythrocytes (RBCs) are the dominant cell type in the blood of the majority of fish and they are important to keep the haemoglobin (respiratory pigment) functionality and also health assessment [20, 21]. In addition to Neger *et al*. [22] observed that, replacing fishmeal with plant protein caused a significant difference in the Hb, haemotocrite, total protein while significant differences were not found in WBCs, RBCs, cholesterol and albumin plasma in *Cyprinus carpio*. The food quality also strongly influences the morphological specifications of blood qualitative and quantitative attributes of haemoglobin in juvenile sturgeons (*Acipenser naccarii)* [23]. The present result also revealed that, the rate of oxygen consumption was gradually decreased with increased the level of SWPM. According to Martin and Fuhrman [24] the metabolism decreased with increase in body weight, mainly because of differential growth rate of many individual body parts in small organisms.

In conclusion, the present experiment showed that the silkworm pupae meal could be utilized in *M. estherae* diets up to 60% instead of the dietary ingredient without any adverse effects on fish growth performance and feed utilization. And supplementation of silkworm pupae meal not only enhanced the growth on *Maylandia estherae* but also reduces the cost of feed formulation.

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