

# Effect of silkworm (Bombyx mori) pupae on the growth and maturation of rainbow shark Epalzeorhynchos frenatum (Fowler, 1934) under captive rearing

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### ABSTRACT

The aim of this study was to determine the impact of replacing fishmeal with silkworm pupae (*Bombyx mori*) meal in the diet of rainbow shark *Epalzeorhynchos frenatum* (Fowler, 1934), on growth and maturation. Four isonitrogenous and isocalorific experimental diets (35% CP) were prepared using silkworm pupae (SWP) replacing fish meal at three different levels *viz.*, 30% (T1), 40% (T2) and 50% (T3) and diet with only fish meal served as control (C). The experimental trail was conducted in hapas, in duplicates, installed in an earthen pond. The fish (average weight - 1.4 g and total length - 5.24 cm) were stocked @10 nos. per hapa. During the experimental period, water quality parameters were monitored routinely. The experimental diets T1, T2 and T3 performed significantly better than diet C (control) in terms of growth performance. Among the four diets, T1 showed significantly higher weight gain (5.18±0.28 g), specific growth rate, SGR (2.54±0.07% day<sup>-1</sup>), protein efficiency ratio, PER (1.50±0.04) and feed conversion ratio, FCR (1.91±0.06) compared to other treatment groups. Among all the diets, highest gonadosomatic index, GSI (14.90%) was obtained in fish fed diet T1. At the end of the experiment, all the experimental diets fed fishes showed similar stages of maturity *i.e.*, late vitellogenic stage. Overall, the results of the present study conclude that SWP can be used as a low-cost animal protein to replace fish meal upto 30% in the diet.

Keywords: Fish meal replacement, Low cost ingredient, Maturational diet, Ornamental fish diet, Rainbow shark, Silkworm pupae

# Introduction

Fishmeal (FM) is one of the major feed ingredient used in the preparation of fish feed due its balanced amino acid composition, high digestibility and palatability, which enhances the uptake, digestion and absorption of nutrients in fish (Miles and Chapman, 2006). However, the steady decline in catches of wild fish and the increased demand for quality aquaculture feed resulted in a rapid decrease in the availability of fishmeal (FAO, 2014). Shortage in fish meal results in high price of fish feed (Rana, *et al.*, 2009; Ayoola, 2010), which demands low cost alternative feed ingredients for sustainable development of aquaculture.

This silkworm (Bombyx mori) pupae (SWP), derived from reeled silk, is used as a protein source in animal feed, due to its high nutritional value and low cost, as well as it is an important fish feed ingredient in the Indo-Pacific region (Hora and Pillay, 1962). Dried SWP is a valuable source of protein (50-71% dry matter DM) and lipid (30%) (Wei

and Liu, 2001). The contents of essential amino acids and protein in silkworm pupae are comparable to fish meal. The fatty acid compositions of lipids of SWP are oleic, palmitic, palmitoleic, stearic, linoleic (24.6% of total lipid), lauric, myristic, linolenic (14% of total lipid) and arachidic acids. The unsaturated fatty acids constitute one-third of the total acids. This SWP has been successfully used as a FM replacement for fish such as catla (Jayaram and Shetty, 1980), rohu (Jayaram and Shetty, 1980; Begun et al., 1994), common carp (Chakraborty et al., 1973; Nandeesha et al., 1990, 2000), mirror carp (Rahman et al., 1996) and jian carp (Ji et al., 2015). Fingerlings of the ornamental fish, silver barb performed better than control, when fed with a diet replacing fish meal with 38% silkworm pupae (Mahata et al., 1994).

Rainbow shark *Epalzeorhynchos frenatum* (Fowler, 1934) belonging to the Cyprinidae family, has good demand in ornamental aquarium fish trade. The huge market demand for this fish has led to large scale breeding

of the species in captivity (Axelrod and William, 1995). However, production of rainbow shark seed throughout the year does not happen as mature broodstock are not available throughout the year. Nutritionally balanced diet is essential for ornamental fish to attain maturation in confined waters. SWP has been observed to be a good and cheap source of protein for ornamental fish (Stahlin, 1957). On the other side, study on SWP inclusion in ornamental fish diets in India is scanty. To our knowledge, there is no research report on replacement of FM with SWP for growth and maturation in the diet of rainbow shark. Hence, the present investigation was carried out to assess the effect of silkworm pupae on the growth and maturation of rainbow shark, *E. frenatum* under captivity.

# Materials and methods

## Experimental fish

Experimental fish (*E. frenatum*), in the weight range of 1.3 to 1.5 g were collected from M/s Udhayam Fish Farm, Tirunelveli, Tamil Nadu, India. The fish were transported in oxygenated HDPE bags to the study location at the Centre for Sustainable Aquaculture, Bhavanisagar, Tamil Nadu, India. They were acclimatised for 2 weeks in hapa installed in an earthen pond. During acclimatisation, the fish were fed a control feed prepared for the experiment (35% protein), at the rate of 5% body weight. Water quality parameters were maintained at optimal level during the acclimatisation period.

## Experimental design

Experiment was conducted in hapas installed in an earthen pond, for a period of 60 days. The experimental setup comprised of 3 sets of treatments and 1 set of control, in duplicates. Rainbow shark fingerlings (average weight of 1.44±0.22 g and total length 5.24±0.33 cm) were stocked at a density of 10 nos. per hapa. Fish were

fed twice a day (09.30 hrs and 16.00 hrs) at the rate of 5% of their body weight.

# Preparation of silkworm pupae meal

Dried silkworm pupae were procured from a private silk reeling industry located in Erode, Tamil Nadu, India. Prior to feed preparation, the outer layer of silk thread (cocoon) was removed from pupae. The cleaned pupae were completely sun dried for two days and ground into powder form which was then stored in an airtight plastic container. The proximate composition of non-deoiled silkworm pupae meal was analysed following standard methods (AOAC, 1995).

### Preparation of experimental diets

Four experimental diets (iso-nitrogenous and iso-caloric pelleted diets), having 35% crude protein were prepared from different combinations of selected feed ingredients (Table 1). Diet with only fishmeal served as control (C) and silkworm pupae (non-deoiled) was used to replace fish meal at 30% (T1), 40% (T2) and 50% (T3) levels. Weighed amounts of the respective ingredients were mixed with sufficient quantity of water to form a dough which was cooked in a pressure cooker for 30 min. The dough was then cooled, mixed with the vitaminmineral mixture and extruded through a pelletiser. The pellets were subsequently dried at about 60°C until the moisture content was reduced to less than 10% (Nandeesha et al., 2000). Proximate composition of the experimental diets and control diets were analysed following standard methods (AOAC, 1995) (Table 2).

# Sampling and water quality analysis

After stocking of experimental fish, the hapas were cleaned once in a week, to ensure proper water circulation, as well as to get rid of algae and other matter clogging the net. During the experimental period,

Table 1. Ingredient composition of formulated experimental diets (% of diet)

	Control	Non-deoiled SWP				
Ingredients		T1	T2	T3		
	С	30%	40%	50%		
Fish meal	30	21	18	15		
SWP	-	9	12	15		
Groundnut oil cake	29	31	32	33		
Corn flour	29	27	26	25		
Cassava starch	6	6	6	6		
Salt	1	1	1	1		
Vitamin premix	0.5	0.5	0.5	0.5		
Mineral premix	0.5	0.5	0.5	0.5		
Yeast	1	1	1	1		
Vitamin E	0.7	0.7	0.7	0.7		
Vegetable oil	2.3	2.3	2.3	2.3		

Diet Ether extract Crude fibre Crude protein Total ash Moisture Gross energy (%)(%) (%) (%) (%)(Kcal kg<sup>-1</sup>) SWP meal (Non-deoiled) 55.63 27.41 7.68 Fish meal 68.21 9.1 1.1 15.1 10.6 5062.56 C 36.27 6.43 3.22 8.06 8.81 4474 T1 34.65 9.07 3.58 7.10 7.73 4456 T2

Table 2. Proximate composition of silkworm pupae meal (SWP) and experimental diets

9.54

11.57

water quality parameters were analysed daily following standard procedures (APHA, 2005). Sampling for growth performance analysis was conducted fortnightly and bio-growth parameters were calculated using the following formulae:

34.77

35.78

Mean weight gain (g) = Mean final weight (g) - Mean initial weight (g)

Specific growth rate, SGR (% day<sup>-1</sup>) = ln final Weight - ln initial Weight / Experimental duration in days X 100

Survival rate, SR (%) = Final numbers of fish obtained/ Initial numbers of fish stocked X 100

Feed conversion ratio (FCR) = Total dry feed fed (g) / Total wet weight gain (g)

Feed efficiency (FE) = Body weight gain (wet weight) / Feed given (dry weight)

Protein efficiency ratio (PER) = Total wet weight gain / Dry weight of protein fed.

# Gonadal maturation study

T3

For studying the gonadal maturation and ovary development, one fish (n=2 per treatment) from each hapa was taken out and sacrificed. The gonads were observed on the 45<sup>th</sup> and 60<sup>th</sup> days of the experiment. The experimental fish were dissected and gonads were taken out with the help of forceps and weighed. Gonadosomatic index (GSI) was calculated using the formula: GSI = Weight of gonad (g) / Body weight of fish (g)  $\times$  100.

The gonad samples collected for histological examination were immediately fixed in 10% neutral buffered formalin. The fixed tissue samples were processed as per Humason (1972) and sections of 5 µm thickness were cut using a microtome (Thermo scientific<sup>TM</sup> 325). Histological sections were then observed under a compound microscope (Nikon Eclipse E200) to assess the developmental stages of gonads.

# Statistical analysis

The results are presented as mean±standard deviation. Data were analysed by one-way ANOVA using the statistical software SPSS 16.0 for windows (SPSS Inc., Chicago, USA). Duncan's multiple range test was used for post-hoc comparison of mean values. The significance level was set at p<0.05.

#### Results

4.02

4.07

Water quality parameters

7.03

6.46

The range of water quality parameters such as temperature, dissolved oxygen, pH, ammonia-N, nitrite-N and phosphate-P were within the optimal range for rearing of rainbow shark (Table 3).

8.05

7.66

4457

4556

## Growth performance

Results of the present study showed significant differences (p<0.05) in growth performance of rainbow shark fed diets with FM replaced by non-deoiled SWP at 30 (T1), 40 (T2) and 50% (T3) and the control (C) diet (Fig. 1). No mortality was observed during the experimental period. The experimental diets T1, T2 and T3 performed significantly (p<0.05) better than diet C (control) in terms of growth performance and feed utilisation by the fishes (Table 4). Significantly higher (p<0.05) mean body weight gain (5.18±0.28 g) was obtained in fish fed diet T1 followed by T2 (3.80±0.42 g), whereas the lowest (p<0.05) mean body weight gain (2.76±0.02 g) was obtained in fish fed on control (C) diet (Table 4). The highest (p<0.05) percentage weight gain (359%) was obtained in fish fed diet T1 followed by T2 (264%), whereas the lowest (p<0.05) percentage weight gain (192%) was obtained in fish fed on control diet (Table 4). SGR of various diets ranged from 1.79 to 2.54% day<sup>-1</sup> (Table 4) and showed significant difference (p<0.05). The highest SGR was observed in fish fed diet T1 followed by T2 and the lowest SGR obtained in fish fed control diet (p<0.05). FCR in this study ranged from 1.91 to 2.8 (Table 4) with significantly lowest (p<0.05) value in fish fed diet T1 (1.91 $\pm$ 0.06), followed by T2 (2.38 $\pm$ 0.25)

Table 3. Water quality parameters (Mean±SD) recorded during

the experimental period	
Parameters	Mean±SD
Temperature (°C)	27.5±0.75
Dissolved oxygen (ppm)	4.75±0.30
pН	$8.1 \pm 0.17$
Ammonia-N (ppm)	$0.07 \pm 0.04$
Nitrite-N (ppm)	$0.55\pm0.14$
Phosphate-P (ppm)	$0.22 \pm 0.08$

Table 4. Growth parameters of rainbow shark fed with diets containing non-deoiled SWP at different levels of FM replacement

Diet	Mean initial weight (g)	Mean final weight (g)	Mean weight gain (g)	Mean initial length (cm)	Mean final length (cm)	Mean length gain (cm)	Percentage weight Gain (PWG)	SGR	FCR	FER	PER
Control		4.20±0.02°	2.76±0.02°		7.79±0.08b	2.55±0.08b	192.01±1.47°	1.79±0.01°	2.85±0.02ª	0.35±0.00b	0.96 <sup>b</sup>
T1	$1.44\pm0.22$	$6.62{\pm}0.28^a$	$5.18{\pm}0.28^a$	5.24±0.33	$8.92{\pm}0.23^a$	$3.68{\pm}0.23^{a}$	$359.38{\pm}19.15^a$	$2.54{\pm}0.07^{\rm a}$	$1.91 \pm 0.06^{b}$	$0.52{\pm}0.02^a$	$1.50{\pm}0.04^{a}$
T2		$5.25{\pm}0.42^{b}$	$3.80{\pm}0.42^{b}$		$8.2 \pm 0.09^{b}$	$2.97{\pm}0.09^{b}$	$264.24{\pm}28.97^{\rm b}$	$2.15{\pm}0.13^{\rm b}$	$2.38{\pm}0.25^{ab}$	$0.42{\pm}0.04^{\text{b}}$	$1.21 \pm 0.12^{b}$
T3		4.92±0.38bc	3.48±0.38bc		8.07±0.19b	2.83±0.19b	241.67±26.52bc	2.05±0.13bc	2.64±0.27b	$0.38 \pm 0.04^{b}$	1.06±0.10 <sup>b</sup>
One way	One way ANOVA (p<0.05)										
Diet	-	0.007	0.007	-	0.009	0.009		0.007	0.029	0.018	0.013

Values are presented as mean±SD

Values in the same column with different superscripts differ significantly (p<0.05) for each parameter.

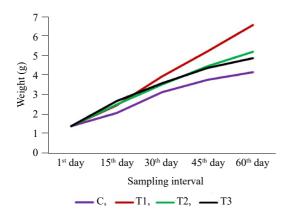


Fig. 1. Growth performance of rainbow shark fed with diets containing non-deoiled and deoiled SWP replacing FM at 30, 40 and 50%

and highest value in control diet (2.85). Better PER value was recorded in fish fed diet T1  $(1.50\pm0.04)$ , followed by T2  $(1.21\pm0.12)$  and the least PER was recorded in diet C (0.96) which is significantly lower (p<0.05) than other treatments (Table 4).

Ovary development and gonadal maturation

The gonadosomatic indices (GSI) recorded ranged from 11.74 to 14.90% observed on  $60^{th}$  day (Table 5). As it is evident, among the treatments, T1 recorded the highest GSI (14.90%) followed by T2 (13.01%). Control fish showed the lowest GSI (11.74%) that was significantly lower (p<0.05) than that of fishes from the treatment groups.

Histological sections of ovary of fishes sampled in the beginning of the experiment showed undeveloped ovary, with numerous primary oocytes, mostly in the perinucleolar phase. Gonad sections of fishes fed control and SWP incorporated experimental diets showed more of mid-vitellogenic ova and few late-vitellogenic ova on the 45th day of the experiment (Fig. 2). On the 60th day of experiment (Fig. 3), control as well as the experimental diets showed similar stages of maturity *i.e.*, late vitellogenic stage characterised by clear ova with distinct nucleus with some of the eggs in maturing phase.

## Discussion

Among the three SWP incorporated diets, 30% inclusion of non-deoiled SWP led to highest weight gain, SGR, PER, FER and better FCR. This outcome is in line with the findings of Mahata *et al.* (1994), who reported that silver barb (*Barbonymus gonionotus*) fingerlings performed better than fish meal-based diet, when fed a diet replacing fish meal with 38% SWP. Similar studies with catla (Jayaram and Shetty, 1980; Nandheesa *et al.*, 1989; Hasan, 1991) and common carp (Jayaram and Shetty, 1980; Nandheesa *et al.*, 1990) revealed 30% fishmeal replacement with SWP, exhibited better growth performances and feed efficiencies.

Results of the present study showed that the highest SGR value was obtained with diet T1 having 30% SWP and the range of SGR recorded in the present study was 1.80 to 2.54% day<sup>-1</sup>. Begum *et al.* (1994) reported that

Table 5. Observations on the weight of the gonads of *E. frenatum* fed with a diet having fish meal replaced with non-deoiled SWP at 30, 40 and 50% respectively on the 60<sup>th</sup> day

Experimental diets	Weight of fish (g)		Weight of gonad (g)		GSI (%)		Mean±SD GSI (%)	
	Replicate1	Replicate2	Replicate1	Replicate2	Replicate1	Replicate2	Mean±SD GSI (70)	
Control (C)	6.8	6.9	0.75	0.86	11.02	12.46	11.74±1.01°	
T1(30% non-deoiled SWP)	8.5	8.20	1.28	1.21	15.05	14.75	14.90±0.21a	
T2-(40% non-deoiled SWP)	8.0	8.2	1.01	1.10	12.62	13.41	$13.01 \pm 0.55^{b}$	
T3 (50% non-deoiled SWP)	8.24	7.95	0.99	0.92	12.01	11.57	$11.79 \pm 0.31^{bc}$	

Values are presented as mean±SD

Values in the same column with different superscripts differ significantly (p<0.05) for each parameter

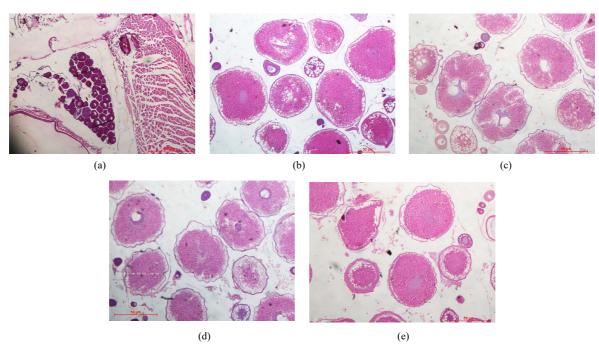


Fig. 2. Histological sections of rainbow shark ovary. (a) Perinucleolar phase observed at the beginning of the experiment; Mid-vitellogenic stage observed in (b) Control; (c) T1, (d) T2 and (e) T3 on the 45<sup>th</sup> day

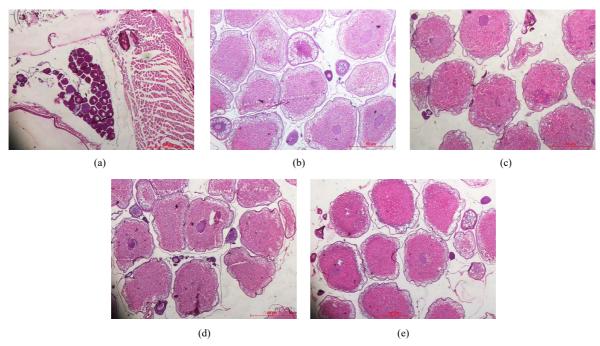


Fig. 3. Histological sections of rainbow shark ovary. (a) Perinucleolar phase observed at the beginning of the experiment. Late-vitellogenic stage observed in (b) Control; (c) T1; (d) T2 and (e) T3 on the 60<sup>th</sup> day

better SGR (1.64% day<sup>-1</sup>) was observed in rohu when fed 50% silkworm pupae diet. Habib *et al.* (1994) found that *Clarias batrachus* fed SWP included diets had higher SGR values (2.06 to 2.12% day<sup>-1</sup>) compared to fish meal, blood worm and poultry byproduct meal supplemented

diets (1.94 to 1.99% day<sup>-1</sup>). Further it was observed that, SWP can be a cheap and effective alternative protein source than poultry byproduct meal and blood meal in the diet of *C. batrachus*. Further, Salem *et al.* (2008) in Nile tilapia (1.2 to 1.42 5 day<sup>-1</sup>) as well as Ji *et al.* (2015) in jian

carp (2.10 to 2.32% day<sup>-1</sup>) also reported similar range of SGR values when fed diets containing different levels of fishmeal replaced with silkworm pupae.

FCR value is used as an important growth determining factor, as feed constitutes the largest operational cost in aquaculture system (Amin *et al.*, 2005). The FCR values observed in the present study ranged from 1.91 to 2.8. However, the values were similar to the FCR values reported by Habib *et al.* (2001) who documented FCR values of 1.95 to 2.70 in *C. batrachus* fed with silkworm pupae-based diet. Nandheesa *et al.* (1988) found that catla-rohu hybrid fed with diets containing SWP had better FCR (2.07 to 2.48). Nandheesa *et al.* (1990) in common carp fry, Jayaram and Shetty (1980) in *L. rohita* fingerlings and Oso and Iwalaye (2014) in *Clarias gariepinus* juveniles also found similar results when fed diets containing various levels of SWP.

Steffens (1981) reported that PER values can be used to evaluate the quality of protein in the diet, those with high PER values, indicate good quality of protein. PER values recorded in the present study were in the range of 1.00 to 1.50. Results showed that fish fed 30% SWP diet had higher PER value compared to other diets. Begum et al. (1994) reported PER value of 1.35 to 1.50 in rohu when fed diet containing different levels of silkworm pupae. Habib et al. (2001) reported PER of 0.95 to 1.25 for diets containing different levels of non-deoiled SWP in *C. batrachus*.

In our study, 40 and 50% replacement of fishmeal with SWP showed significantly better growth performance compared to the control diet. Earlier studies with rohu (Begun et al., 1994) and common carp (Nandheesa et al., 2000) have shown the best growth performance upto 50% FM replaced with silkworm pupae. The present study also showed that weight gain in fishes fed diets with 40 and 50% replacement of fishmeal with SWP was comparatively less than fishes fed 30% SWP diet. Increase in the dietary SWP level resulted in significant decrease in growth rate. Similar findings were reported by Ji et al. (2015) in jian carp and Salem et al. (2008) in Nile tilapia. This may primarily be due to high levels of chitin in SWP (5-15%) which is poorly digested by fish (Cummins et al., 2017). Further studies in fish, showed that increased substitution or replacement of FM by insect meal simultaneously increase the chitin level and affected the digestibility of proteins (Longvah et al., 2011) and lipids (Kroeckel et al., 2012). Replacement of FM by 30% non-deoiled SWP showed higher protein digestibility in catla (Umalatha et al., 2018), common carp (Jayaram and Shetty, 1980; Nandheesa et al., 1990; Gangadhar et al., 2017) and Labeo firmbriatus (Gangadhar et al., 2017), compared to fishes fed control diet.

In the present study, ovary development on the 60<sup>th</sup> day of the experiment, in the control and treatments showed similar stage of maturity *i.e.*, late vitellogenic stage. Resutls of the resent study clearly indicated that replacement of fishmeal by silkworm pupae meal will not affect maturation of the fish. In short, all the experimental diets used in the experiment helped in development of ovary and maturation of ova.

From results of the present study, it could be concluded that SWP can be used to replace FM in the diets of rainbow shark. However, replacement levels of 30% was found to be more efficient. Increasing levels of incorporation of SWP in the diet leads to reduced growth and increased FCR. Further, SWP is cheaper than FM and it would definitely provide a cheap alternative protein source to improve the yield for farmers.

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