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REPLACEMENT OF COSTLY FISH MEAL BY SILKWORM PUPAE IN DIET OF MIRROR CARP (*CYPRINUS CARPIO* L.)

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Four isonitrogenous (38% protein) diets were prepared using fish meal, silkworm pupae (*Bombyx mori*), mustard oil cake (*Brassica juncea*) and maskali bran (*Phaseolus mungo*) as dietary protein sources. Silkworm pupae (SWP) was included in the diets at 40, 30 and 20% of dietary level of protein in the diet 2, 3 and 4 respectively. The dietary protein from mustard oil cake and maskali bran were kept constant. These diets were tested in cistern tank for 77 days to evaluate the low-cost silkworm pupae meal in the diet of mirror carp (*Cyprinus carpio* L.) and growth response of fish fed these diets were compared to fish fed a fish meal based control diet (diet 1). Diet 2 and 3 showed significantly ($P < 0.05$) higher growth performance followed by diet 4. The control diet without silkworm showed poorest growth performance. Body lipid and protein content of fish were found to increase in fish fed higher levels of SWP based diets. The results of the study indicate the possibility of replacing the costly fish meal with silkworm pupae in formulating low-cost diet for mirror carp.

Key words: Fish meal, Silkworm pupae, Diet of mirror carp.

Introduction

Supplemental feeds is an essential prerequisite for intensive or semi-intensive fish culture under captivity to have increased production. Formulation of a suitable feed considers not only the selective presence of different ingredients but also the qualitative aspects of those ingredients. Most important factor is to find source of protein. Presently fish meal has been used as the chief source of protein in supplemental feeds in Bangladesh. However, the cost of fish meal has been a matter of great concern in developing supplemental feed with required amount of protein. The present study was, therefore, conducted to search for an effective alternative for fish meal. Silkworm pupae is an important fish feed in the Indo-Pacific region, particularly in China and Japan [1]. About 48% of total composition of silk goods are known to be pupae which are obtainable four times in a year [2]. Compared to fish meal, silkworm pupae is a low cost ingredient and rich both in protein and lipid [3]. Two large silk industries and 360 cottage industries have been working on silk in northern districts of greater Rajshahi producing 50 metric tons of silkworm pupae per year [3]. Besides, innumerable cottage silk industries are being rapidly developed through out the country under the auspices of non-govt. agencies (NGOs). Although no statistics available on how much pupae is produced altogether, nevertheless a formidable amount would be available in the country which go to waste now. Chakrabarty *et al.* [4] Jayachandran and Raj [5], Jayaram and Shetty [6], Nandeesa *et al.* [7] studied the possibility of using silkworm pupae in various indigenous and exotic fish. Recently, Hasan [8] and Mahata [2] used silkworm pupae as an effective dietary protein source for *Catla catla* and *Puntius gonionotus* respectively as a replacement of fish meal. Being a fast growing fish and having high market value, mirror carp has been chosen for the present study.

Materials and Methods

Diet ingredients. 'A' grade fish meal was bought from Bangladesh Fisheries Development Corporation, Chittagong. Mustard oil cake (*Brassica juncea*) of expeller type was collected from a local ghani mill and was finely ground. Maskali bran (*Phaseolus mungo*) was procured from a local market. Silkworm pupae (*Bombyx mori*) was purchased from Rajshahi silk industry. All ingredients were sieved through a 0.5 mm sieve to produce a powdery form. Proximate composition of all ingredients was analyzed by AOAC [9] method prior to formulation of diets (Table 1).

Preparation of diets. Four isonitrogenous (38% protein) fish diet; group 1, 2, 3 and 4 were prepared by mixing finely ground indigenous ingredients in different proportions to obtain 38% protein, 11-12% lipid and 25-30% soluble carbohydrate. In the control diet (diet 1)fish meal was used as the sole source of protein. SWP was included in various levels to replace 20, 30 and 40% of fish meal protein in treatment group 4, 3 and 2 respectively (Table 2). The contribution of dietary protein from mustard oil cake and maskali bran was kept constant in test diets. The bite-sized (4mm) diets were prepared according to the methods of Jauncey [10]. A stock of each 15 days per installment were prepared and stocked

tightly in an air tight container. The prepared diets were then analyzed for proximate composition (Table 2).

Experimental system and animal. The experiment was conducted in 4 cisterns each 789 × 112 × 82 cm in size for 11 weeks. Each cistern was divided into three equal parts to have twelve chambers by partitioning with galvanized wire 0.3 cm meshes. Each such small part was used as a replicate. There were 10 fish measuring 42 ± 2g per replicate irrespective of sex. The treatment groups (4 diets) with three replicates were randomly distributed to the chambers. The fish were fed with the experimental diets two times daily (morning 9.00 am and evening 5.00 pm) at the rate of 3% of the body weight for the 1st 5 weeks and 2% of the body weight for the rest of the experimental period. The fish were bulk weighed weekly to adjust the ration for the next week. The water level in each cistern was maintained at about 40 cm height. The water in each cistern was also changed every day using a continuous flow of tap water from main water supply of FRI hatchery for 4-6 hrs which allowed about total exchange in each cistern. Aeration of cistern water was made with a supply of airflow. Water parameters for temperature, pH, dissolved oxygen and carbon di-oxide were measured weekly with a Hack Kit.

TABLE 1. PROXIMATE COMPOSITION OF DIETARY INGREDIENTS (% OF DRY MATTER BASIS).

Ingredients	Components					
	Dry matter	Crude protein	Crude lipid	Crude fibre	Ash	Nitrogen free extract (NFE)*
Fish meal	86.9	59.64	11.2	2.7	22.8	3.7
Silkworm pupae	92.04	55.56	23.2	6.2	7.3	10.8
Mustard oil cake	85.5	30.34	13.4	12.1	9.7	34.4
Maskali bran	87.07	18.89	0.55	22.83	7.47	50.26
Barley	86.87	0.93	0.21	-	-	98.86

*NFE = Nitrogen free extract calculated as 100% (moisture + crude protein + crude lipid + ash + crude fibre)

TABLE 2. FORMULATION AND PROXIMATE COMPOSITION OF THE EXPERIMENTAL DIETS (% OF DRY MATTER BASIS).

Ingredients	Diet No.			
	1(control)	2	3	4
Formulation:				
Fish meal	63.74	19.12	25.49	31.87
Silkworm pupae	-	28.97	21.73	14.48
Mustard oil cake	-	25.05	25.05	25.05
Maskali bran	-	20.11	20.11	20.11
Soybean oil	4.00	-	-	1.00
Vitamin premix*	1.00	1.00	1.00	1.00
Mineral premix*	1.00	1.00	1.00	1.00
Starch (Barley)	30.26	4.75	5.62	5.49
Proximate composition:				
Dry matter	93.94	94.96	95.88	95.77
Crude protein	38.05	37.70	38.27	38.50
Crude lipid	10.90	11.80	11.40	11.56
Crude fibre	2.51	9.01	9.67	9.10
Ash	14.18	10.40	10.56	10.51
NFE	28.30	26.05	25.98	25.45
Gross energy** (Kcal/g)	4.24	4.22	4.21	4.19
PE ratio***	89.74	89.34	90.90	91.05

* = According to Akhter [20], ** = According to Jauncey and Ross [11], *** = Protein to energy ratio in mg protein/Kcal of total energy.

Analytical procedure. To obtain isonitrogenous level and calorie value of fish diets in the dietary groups, the proximate composition of the dietary ingredients, diets and fish carcass were analyzed in triplicate according to AOAC [9]. Gross energy obtained from carbohydrate, lipid and protein was calculated according to Jauncey and Ross [11]. Analysis of experimental results was done as Castell and Tiews [12]. Quality of fish diets in respect to growth rates was analyzed statistically by the multiple range method of Duncan [13].

Results and Discussion

The proximate composition of the dietary ingredients is shown in Table 1. The non-conventional dietary ingredient SWP contained 55.56% protein which is near about the protein content of fish meal (59.64%). The lipid content of SWP is more than double (23.23%) that of fish meal (11.22%). Diet formulations are shown in Table 2. Thirty eight percent protein levels were maintained in each diet. Diet group I contained only fish meal as the source of protein which was treated as control. In diet group 2, 3 and 4, fish meal proteins were replaced by SWP protein at the rate of 40, 30 and 20% respectively. The proximate composition of various experimental diets is shown in Table 2. Crude protein lipid and NFE content hardly varied among the test diets. In diet 1 (control), ash content (14.18%) was high and fibre content (2.51%) was low compared to other diets. Gross energy level hardly varied from 4.19 to 4.24 kcal/g among the formulated diets.

Growth responses and food utilization efficiency of the fish during the 11 weeks experiment in concrete cistern are shown in Table 3 and Fig. 1. As shown in Fig. 1, the highest growth was obtained with the fish fed diet 2 followed by diet 3 and 4 and poorest growth with the fish fed on diet 1. The fish which fed on diet 2 containing 28.97% SWP showed significantly ($p < 0.05$) highest weight gain followed by diet 3 and 4 (Table 3).

The range of water quality parameters recorded during the experimental period: temperature 22.3-28.0°C, dissolved

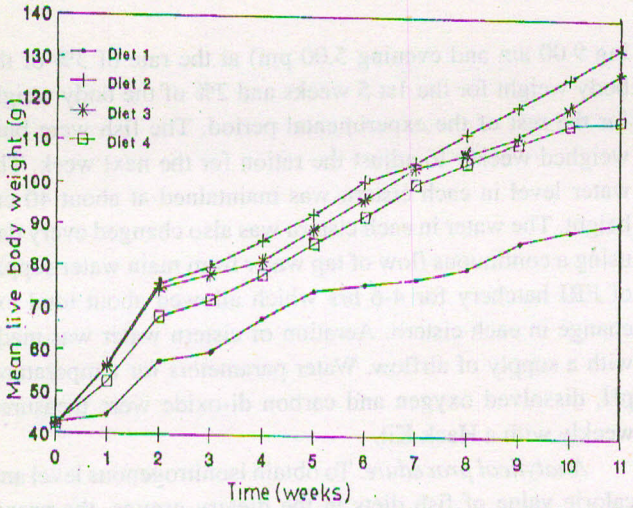

Fig. 1. Growth responses of mirror carp fingerlings fed experimental diets for 11 weeks.

oxygen 3.92-5.4 mg/l, pH 6.8-8.2 and carbon di-oxide 6.8-10.6 mg/l. Dissolved oxygen level was bit low because underground water used in the cistern was almost free from oxygen. The reported level was maintained by continuous airflow. Mahata [2] had the similar levels of these parameters during conducting trials in fibre glass troughs.

The best food conversion ratio (FCR) value was achieved by the fish fed diet 2 (2.20) and the worst FCR value was with the fish fed diet 1 (3.07). However, there were no significant differences ($p>0.05$) between the FCRs of the fish fed on diet 2 and 3 but significant differences between that of diet 3 and 4.

The protein efficiency ratio (PER) and apparent net protein utilization (ANPU) values followed the same trend. There were no significant differences ($p > 0.05$) of PER and ANPU values between diet 2 and 3 and the diet of 3 and 4 respectively. The highest value of PER and ANPU was obtained with the fish fed diet 2 followed by diet 3 and 4.

Carcass composition study (Table 3) showed fish fed diet 1 had the highest moisture (75.16%) content and lowest lipid (5.25%) content whilst fish fed diet 2 had the lowest moisture (74.26%) and highest lipid (5.85%) content. The crude protein content ranged from 15.07 to 16.04% and fish fed diet 2 had the highest crude protein content (16.04%). The carcass ash content varied between 2.27 to 2.57%.

The growth response of the fish fed the experimental diets revealed that the fish fed diet 2 containing 28.97% SWP replacing 40% of total dietary fish meal protein showed the best growth performance among the experimental diets following diet 3 containing 21.73% SWP replacing 30% of fish meal protein. The fish meal based control diet (diet 1) showed poor growth performance.

Our study is consistent with that reported by Jayachandran and Raj [5] for common carp fed SWP diets. Furthermore, Nandeesha *et al.* [7] reported that silkworm pupae showed higher growth performance on mirror carp when the fish meal was totally replaced in the diet which ultimately proved the superior quality of SWP than that of fish meal. The poor growth performance of diet 1 is due to the low fibre content, comparatively lower lipid content and poor palatability of the diet.

Silkworm pupae oil in the diet is postulated to be an appetite stimulant and attractant to the fish [14,15]. Tsushima and Ina [15] and Nandeesha *et al.* [16] observed poor growth response of common carp fed diets containing 15, 20 and 25% of deoiled SWP. In this study diet 2 containing highest amount of SWP oil (6.73%) compared to diet 3 and 4 showed best growth performance although not statistically ($p > 0.05$) significant. This clearly indicate the importance of silkworm pupae oil in the fish diet and support the view that higher levels of SWP oil in the diet 2 could possibly the reason for better growth in comparison with other diets.

It is also remarkable that inclusion of SWP in diet 3 and 4 improved the growth of fish compared to that of fish meal based control diet which clearly indicate the significant role of SWP on the higher growth performance of mirror carp.

The poor growth response in fish fed diet 1 containing

TABLE 3. GROWTH, FOOD UTILIZATION AND CARCASS COMPOSITION OF MIRROR CARP FED EXPERIMENTAL DIETS FOR 11 WEEKS

Mean values	Diet No.				
	1 (control)	2	3	4	SE*
Initial body weight (g)	42.48a**	42.50a	42.05a	42.46a	0.328
Final body weight (g)	91.46d	132.71a	127.29b	115.79c	0.779
Weight gain (g)	48.62d	90.21a	85.25b	73.33c	0.793
% Weight gain (g)	113.52b	212.33a	202.76b	172.72c	2.708
Specific growth rate (SGR %day)	0.99d	1.48a	1.44b	1.31c	0.013
Food conversion ratio (FCR)	3.07a	2.20cd	2.22c	2.50b	0.053
Protein efficiency ratio (PER)	0.87c	1.20a	1.18ab	1.13b	0.345
Apparent net protein utilization (ANPU%)	13.60c	20.41a	19.54ab	16.82b	0.398
Carcass Composition:					
	Initial (all fish)				
Moisture	75.57	75.16	74.26	74.47	74.84	0.054
Crude protein	14.16	15.07	16.04	15.80	15.36	0.028
Crude lipid	4.87	5.25	5.85	5.54	5.42	0.020
Ash	2.25	2.57	2.27	2.42	2.49	0.028

* Standard error of treatment mean calculated from residual mean square in the analysis of variance.

** Figures in the same row having the same super scripts are not significantly different ($p > 0.05$).

fish meal is also probably due to the low fibre (2.51%) content of the diet compared to other diets (9%). The diets 2, 3 and 4 containing 9% crude fibre showed better growth. This is in agreement with the recommended value of crude fibre between 8 to 10% formulated fish feed [11]. In studies with channel cat fish, Leary and Lovell [17] proposed that cellulose (crude fibre) might increase the residence time for the purified, readily soluble ingredients of the diets in the digestive tract, thus allowing an increased uptake of nutrients. This might partly explain the decreased efficiency of protein utilization in fish receiving diet 1. Buhler and Halver [18] reported that the addition of 9% cellulose to diets for chinook salmon resulted in improved protein utilization compared to a control diet without fibre.

The experimental diets (2, 3 and 4) containing highest level of SWP increased the body lipid and protein content of the fish and decreased the moisture content to some extent. Similar observations are reported by Nandeesha *et al.* [19].

The results of this study indicate the possibility of replacing fish meal with silkworm pupae. As fish culture is not concerned only with growth, but also with production cost, the low-cost of SWP (Tk. 10/kg) compared to fish meal (Tk. 25/kg) can be advantageous in developing cheaper but high quality diets for carp (mirror carp). Both fish farmers and fish producers in Bangladesh could benefit from this type of research to produce maximum fish with minimum cost.

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