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# Growth Response and Flesh Quality of Common Carp, *Cyprinus carpio* Fed with High Levels of Nondefatted Silkworm Pupae

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## Abstract

Nondefatted silkworm pupae, a major by-product of the sericulture industry in India is rich in protein and fat. A growth trial was conducted over a period of 90 days to study the usefulness of pupae as a source of protein and energy in the diet of common carp, *Cyprinus carpio*. Results indicated that the pupae could be used to completely replace fishmeal and could be included up to 50% in the diet. There was no significant difference in the final weight gain, food conversion ratio, and protein efficiency ratio of fish fed with fishmeal based control diet and diets incorporated with 30, 40 or 50% pupae. However, the net protein retention was found to increase with increasing levels of pupae in the diet. Ribonucleic acid/deoxyribonucleic acid (RNA/DNA) ratio was highest in fish fed 50% pupae. Protein deposition in the carcass increased, while fat content decreased as pupae content in the diet increased. Protease enzyme activity was higher in pupae fed fish. Organoleptic evaluation of the fish indicated no significant difference between any of the treatments, either in the overall quality or individual attributes such as odor, texture, and flavor implying that pupae can be safely used up to 50% without hampering the growth and meat quality.

## Introduction

Common carp is one of the most widely cultured species in India. This species is omnivorous, hardy, tolerant of wide fluctuations in environmental conditions and is preferred for culture by many farmers. A wide variety of locally available ingredients are used as feed for common carp. Due to the rapid expansion of sericulture in several parts of India, particularly in the southern states, there is ample scope for the utilization of silkworm pupae, a

major by-product of this industry, as a fish feed ingredient. Studies on the usefulness of silkworm pupae as feed for Indian major carps and common carp fingerlings have shown that it is more suitable than mustard oil cake and rice bran as feed (Chakrabarthy et al. 1973). In an earlier study conducted by Nandeesha et al. (1990), it was shown that feeding common carp with diets containing up to 30% pupae resulted in progressive increase in growth with the increasing level of pupae as compared to a fishmeal based 30% protein diet, and the highest weight was recorded at 30% of pupae incorporation. The present study was conducted to investigate the possibility of using silkworm pupae at higher levels.

## Materials and Methods

Three diets were formulated by incorporating powdered dry pupae at 30% (30% P), 40% (40% P) and 50% (50% P), completely replacing fishmeal. In addition to fishmeal, other ingredients, particularly groundnut cake level was altered to balance the protein level (Table 1). The diet with fishmeal served as the control. The nutrient and energy levels were kept almost constant in all the diets using cod liver oil as an additional energy source and sugarcane pith powder as a non-energy filler. Weighed amounts of the respective ingredients were mixed with sufficient quantity (800 ml $kg^{-1}$) of water to form a dough which was cooked in a pressure cooker for 30 minutes. The dough was then cooled, mixed with vitamin-mineral mixture, and extruded through a pelletizer. The pellets were subsequently dried at about 60°C till the moisture content was reduced to less than 10%.

Advanced fry of common carp (0.43 $\pm$ 0.03 g) were stocked in 25 $m^3$ (5 x 5 x 1 m) cement cisterns at 12,000 $ha^{-1}$. For each treatment, duplicate unfertilized cement cisterns were used. Fish were fed once daily with the respective test diets at 5% body weight during the entire culture period. Fish were sampled at fortnightly intervals by collecting at least 50% of the fish using a drag net and the feed quantity was adjusted based on the increased body weight. During every fish sampling, water quality was also measured for pH, dissolved oxygen, free carbon dioxide and alkalinity, following APHA (1985) procedures.

Upon termination of the experiment, three fish were taken from each of the replicate cisterns and dried at 80°C to a constant weight to determine moisture content. The dried carcass of each treatment group was pooled and ground and proximate composition was determined following AOAC (1975) procedures. Five to six fish, randomly collected from each treatment and the control were sacrificed by pithing. They were first dissected on ice, then the alimentary canal and hepatopancreas were cleaned, removed and thoroughly washed with chilled physiological saline. Portions of the alimentary canal were cleaned by pumping chilled saline into them using a syringe. The hepatopancreas and the intestines were then homogenized separately with 10 volumes of chilled saline to one part by weight of the dissected tissue to make a homogenate of the extract. Each homogenate was then centrifuged at 10,000

rpm for 10 min. in a refrigerated centrifuge at 4°C to remove cell debris and nuclei. The supernatent was collected and stored at less than 0°C until further analyses. This supernatent was used to estimate the activity of digestive enzymes, namely amylase (Sumner 1947), protease (Kunitz 1947), and lipase (Naher 1974). Nucleic acid contents of muscle samples were analyzed following the method of Schneider (1957). In addition, fish muscle samples were also analyzed for organoleptic quality in both raw and cooked (in 1.5% salt solution for 3 min.) forms by a group of 13 trained panelists. Data pertaining to overall quality was analyzed using the method recommended by Jayaram et al. (1980), while the individual attributes were analyzed following the Krammer and

Table 1. Ingredient proportion and proximate composition of the diets.

| | Diets | | | |
|---|---|---|---|---|
| | Control | 30% P | 40% P | 50% P |
| Ingredient (%) | | | | |
| Fish meal | 25 | - | - | - |
| Silkworm pupa | - | 30 | 40 | 50 |
| Groundnut cake | 23 | 27 | 12 | - |
| Rice bran | 34 | 25 | 30 | 20 |
| Cod liver oil | 9 | 3 | 1 | - |
| Tapioca flour | 8 | 10 | 14 | 20 |
| Sugarcane pith powder* | - | 4 | 2 | 9 |
| Vitamin and mineral mixture** | 1 | 1 | 1 | 1 |
| Proximate composition (%) | | | | |
| Moisture | 4.17 | 4.32 | 4.33 | 3.86 |
| | (0.15) | (0.11) | (0.08) | (0.10) |
| Crude protein | 28.12 | 27.68 | 27.56 | 27.51 |
| | (0.06) | (0.04) | (0.35) | (0.31) |
| Fat | 14.46 | 14.63 | 14.98 | 15.47 |
| | (0.21) | (0.04) | (0.13) | (0.08) |
| Ash | 15.75 | 13.69 | 14.90 | 12.15 |
| | (0.10) | (0.37) | (0.16) | (0.23) |
| Crude fibre | 13.58 | 11.15 | 12.00 | 15.10 |
| | (0.11) | (0.35) | (0.10) | (0.20) |
| NFE | 23.38 | 28.53 | 26.23 | 25.91 |
| Energy (kJ $g^{-1}$) | 16.37 | 17.22 | 16.94 | 17.06 |

*Used only as a filler.

**Supplevite-M (each 250g provides vit.A-500,000 IU, vit $D_3$-100,000 IU, vit.$B_2$-0.2g, vit.E-75 units, vit.K-0.1 g, calcium pantothenate-0.25 g, nicotinamide-1 g, vit.$B_{12}$-0.6 mg, choline chloride 15 g, calcium 75 g, manganese 2.75 g, iodine 0.1 g, iron-0.75 g, zinc-1.5 g, copper-0.2 g, cobalt-0.045 g).

Figure in parentheses indicate standard deviation.

Proximate composition and price of ingredients -

Fish meal – 8.44% moisture (M), 64.01% crude protein (CP), 3.82% Fat (F) and 20.57% ash (A), price $kg^{-1}$ in Rs. (P) = 20.00.

Silkworm pupae – 5.14% M, 50.02% CP, 27.85% F, 4.93% crude fibre (CF) and 10.69% A, P=2.50

Groundnut cake – 8.34% M, 39.37% CP, 6.83% F, 8.00% CF and 9.07% A, P=10.00.

Rice bran- 8.22% M, 10.04% CP, 5.20% F, 30.24% CF and 18.05% A, P=4.00

Tapioca flour – 9.46% M, 2.80% CP, 0.33% F, 2.50% CF and 1.30% A, P= 15.00

Twigg (1970) method. Analysis of variance followed by Duncan's multiple range test (Duncan 1955) were used for the statistical analysis of all the other data.

## Results

The water quality parameters in different treatments were within the optimal range for carp. Temperature ranged from 26 to 29°C, with oxygen fluctuating between 5.63 to 8.83 ppm. Water pH ranged from 7.90 to 8.70 and total alkalinity from 33.20 to 69.70 mg $CaCO_3 \cdot L^{-1}$.

The growth trend observed during the 90 - day culture showed no significant difference between any of the treatments (Table 2) at 5% level of significance. No differences were also noted in specific growth rate, food conversion ratio and protein efficiency ratio. However, interestingly the net

Table 2. Growth indices and carcass composition of common carp fed different diets.

| Parameter | Diets | | | |
|---|---|---|---|---|
| | Control | 30% P | 40% P | 50% P |
| Final average weight (g) | 40.26 | 40.55 | 38.88 | 41.17 |
| | (1.17) | (1.29) | (1.24) | (0.93) |
| Net weight gain (g) | 39.83 | 40.12 | 38.45 | 40.74 |
| | (1.17) | (1.29) | (1.24) | (0.93) |
| Specific growth rate (%) | 4.82 | 4.82 | 4.78 | 4.85 |
| | (0.06) | (0.06) | (0) | (0.04) |
| Final average length (cm) | 11.98 | 12.10 | 11.79 | 11.72 |
| | (0.78) | (0.76) | (0.78) | (0.92) |
| Net length gain (cm) | 8.84 | 8.96 | 8.65 | 8.58 |
| | (0.78) | (0.76) | (0.01) | (0.92) |
| Food conversion ratio | 1.55 | 1.54 | 1.61 | 1.52 |
| | (0.07) | (0.09) | (0.01) | (0.05) |
| Protein efficiency ratio | 2.26 | 2.31 | 2.24 | 2.36 |
| | (0.12) | (0.13) | (0.03) | (0.08) |
| Net protein retention (%) | $13.47^{a}$ | $14.79^{b}$ | $18.85^{c}$ | $20.11^{d}$ |
| | (0.45) | (0.56) | (0.39) | (0.43) |
| Muscle RNA/DNA | $6.82^{a}$ | $7.15^{a}$ | $6.69^{a}$ | $8.29^{b}$ |
| | (0.26) | (0.28) | (0.05) | (0.51) |
| Survival (%) | 80.00 | 81.66 | 85.00 | 80.00 |
| | (4.94) | (2.12) | (2.12) | (4.94) |
| Carcass composition (%) | | | | |
| Moisture | 72.75 | 73.50 | 73.94 | 74.04 |
| | (0.64) | (0.70) | (0.40) | (0.73) |
| Crude protein | $13.75^{a}$ | $14.00^{b}$ | $14.63^{c}$ | $15.20^{d}$ |
| | (0.08) | (0.17) | (0.06) | (0.07) |
| Fat | 10.15 | $8.78^{c}$ | $7.99^{b}$ | $7.25^{a}$ |
| | (0.09) | (0.01) | (0.03) | (0.12) |
| Ash | $1.96^{a}$ | $2.06^{ab}$ | $2.15^{bc}$ | $2.27^{c}$ |
| | (0.04) | (0.05) | (0.03) | (0.07) |

Figures in the same row having the same superscript and without superscript are not significantly different at 5% level.
Figures in parentheses indicate standard deviation (n = 2 for growth indices and n = 3 for proximate composition).

protein retention was found to increase with increasing levels of pupa in the diet, the highest being at 50% incorporation. The RNA/DNA ratio was higher in fish fed pupae incorporated diets, the highest being in 50% pupae treatment.

The proximate composition analysis showed no difference in the moisture content of fish carcass. While the protein and ash contents remained low in control, a progressive increase in their percentage was noticed with increasing level of pupa. Consequently, the fat percentage decreased with increasing pupae incorporation (Table 2).

Among the digestive enzymes analyzed, the activity of protease was higher in fish fed with 40% and 50% pupae based diets, in both intestine and hepatopancreas (Table 3). Amylase and lipase activities were generally higher in fish fed control diet. The results of organoleptic evaluation showed no difference ($P > 0.05$) in respect to various attributes between the treatments. However, the average score obtained for fish fed 50% pupae incorporated diet was generally higher as compared to other treatments (Table 4).

Table 3. Digestive enzyme activity* in the gut of common carp fed silkworm pupae incorporated diets.

| | Protease | | Amylase | | Lipase | |
|---|---|---|---|---|---|---|
| | Intestinal | Hepatopancreatic | Intestinal | Hepatopancreatic | Intestinal | Hepatopancreatic |
| Control | $1.30^{a}$ | $1.09^{a}$ | $0.94^{c}$ | 4.04 | $0.25^{c}$ | $0.28^{b}$ |
| | (0.02) | (0.04) | (0.02) | (0.07) | (0) | (0) |
| 30% P | $1.18^{a}$ | $0.85^{a}$ | $0.24^{a}$ | 3.83 | $0.12^{b}$ | $0.03^{a}$ |
| | (0.01) | (0.05) | (0.02) | (0) | (0) | (0) |
| 40% P | $1.80^{b}$ | $1.57^{b}$ | $0.50^{b}$ | 3.63 | $0.07^{a}$ | $0.07^{a}$ |
| | (0.02) | (0) | (0.03) | (0.04) | (0) | (0) |
| 50% P | $1.86^{b}$ | $2.43^{c}$ | $0.59^{b}$ | 4.31 | $0.02^{a}$ | $0.09^{a}$ |
| | (0.02) | (0.01) | (0.03) | (0) | (0) | (0) |

Figures in parantheses indicate standard deviation (n = 3).
Figures in the same column with same superscript are not significantly different at 5% level.
*Enzyme activity is expressed as µ moles of product liberated per minute per 10 mg protein at 28°C.

Table 4. Organoleptic attributes (n = 13) of common carp fed silkworm pupae incorporated diets.

| Diet | Overall quality | | Odor of flesh | | Texture of flesh | | Flavor |
|---|---|---|---|---|---|---|---|
| | Raw | Cooked | Raw | Cooked | Raw | Cooked | Cooked |
| Control | 3.55 | 3.10 | 3.31 | 3.31 | 3.38 | 3.15 | 3.00 |
| 30% P | 3.38 | 3.12 | 3.31 | 3.31 | 3.69 | 2.92 | 3.08 |
| 40% P | 3.31 | 2.92 | 3.31 | 3.15 | 3.46 | 2.62 | 2.92 |
| 50% P | 3.31 | 3.15 | 3.38 | 3.46 | 3.54 | 2.85 | 3.15 |

Figures in the same column are not significantly different at 5% level.

## Discussion

The fishmeal based control diet with approximately 30% protein has been used for the experimental culture of carp at this Institute for many years (Nandeesha et al. 1995; 1998; 1999), hence it was used as the reference diet in the present study.

Good growth of common carp at all levels of pupae incorporation clearly indicates that pupae can be incorporated even up to 50% level without any adverse effect. Earlier studies with common carp (Nandeesha et al. 1990), catla and rohu (Jayaram and Shetty 1980a) have shown the best growth of carps at 30% pupae inclusion, being the highest level tried. The insignificant difference in growth, food conversion ratio and protein efficiency ratio observed in the present study as compared to control treatment even up to 50% pupae incorporation clearly indicates that fishmeal (costing Rs. 20 $kg^{-1}$) and even groundnut cake (Rs. 10 $kg^{-1}$) can be replaced by the cheaper silkworm pupae (Rs. 2.50 $kg^{-1}$) and thereby reduce the cost of fish production. Begun et al. (1994) recorded significantly better specific growth rate, food conversion ratio, and protein efficiency ratio for rohu fed with a diet, 50% of its protein being contributed by silkworm pupae compared to a fishmeal based diet. The progressive increase in nitrogen retention with increasing levels of pupae indicates the efficient utilization of pupal fat as an energy source in the present study. The RNA/DNA ratio, a reliable indicator of protein synthesis and thus growth (Khan and Jafri 1991; Steinhart and Eckman 1992) was also found to be higher in fish fed with pupae incorporated diets.

Proximate analyses of the carcass strongly reveal that the dietary pupae protein has been utilized by the fish, resulting in the accretion of carcass protein content. It is reported that the body lipid concentration of fish is positively related to the level of dietary lipid and energy (Takeuchi et al. 1978). On the contrary, the reduction in the fat content of common carp fed with increasing amount of pupae suggests the utilization of pupal fat as an energy source in common carp. Pupa oil is rich in short chain unsaturated fatty acids and has been found to be an excellent energy source in the diet of common carp (Nandeesha et al. 1999). The inverse relationship betweeen moisture and fat as observed in the present study is reported in several earlier studies (De Silva et al. 1991).

Diet is known to influence the organoleptic quality of fish (Lovell and Sackey 1973; Spinelli et al. 1979). Earlier studies with Indian major carps have also shown no adverse effect of silkworm pupae on organoleptic quality (Jayaram et al. 1980b; Nandeesha et al. 1990). Fat content of raw pupa is known to induce off-flavor (Hora and Pillay 1962; Spinelli et al. 1979) and unpleasant taste (Rath 1993) when incorporated at high levels and fed to fish for a long period. No such observation was recorded in the present study and this indicates that pupa can be used up to 50% without any adverse effect.

The results of the present study demonstrate that nondefatted pupae can be profitably used even up to 50% in carp diet by completely replacing fish meal and even by reducing the level of other costly inputs like groundnut cake.

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