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Silkworm Pupa Oil and Sardine Oil as an Additional Energy Source in the Diet of Common Carp, *Cyprinus carpio*

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Abstract

A 90-day field experiment was conducted on common carp, *Cyprinus carpio* to study the usefulness of silkworm pupa oil as an energy source in comparison with sardine oil. Dietary administration of three levels of pupa oil and sardine oil, *viz.*, 3, 6 and 9% was used to enrich a fishmeal based diet while a diet without oil enrichment served as control. Fish growth increased significantly with the increasing level of both pupa and sardine oils, while the control diet led to the lowest growth. There was no significant difference in the final mean weights attained under pupa and sardine oil treatments at the same level of incorporation. Specific growth rate, feed gain ratio, and biomass production improved significantly with increasing level of oil incorporation. The percentage increase in biomass as compared to control was nearly 50% at 9% addition of either oils. Organoleptic evaluation showed no adverse effect of oils on the flesh quality. The study demonstrated not only the usefulness of pupa oil, which is rich in unsaturated fatty acids (n-3), as an equally potent energy source as sardine oil, but also the possibility of using additional fat in the diet to economically promote carp growth.

Introduction

The culture of Indian major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) and exotic carps (*Ctenopharyngodon idella, Hypophthalmichthys molitrix*, and *Cyprinus carpio*) is expanding at a rapid rate in India. With the intensification of aquaculture of these cyprinids, the need for evolving cost-effective diets is recognised as very important. The high cost of fishmeal has necessitated the search for alternate protein sources and non-defatted silkworm pupa has been found to be one of the best substitutes to fishmeal in the diets of several carp species (Shetty and Jayaram 1978; Nandeesha et al. 1990a). However, in recent years, oil is often extracted from pupa and two products *viz.*, defatted pupa and pupa oil are commercially available. Earlier studies conducted with defatted pupa indicated its unsuitability as a complete replacement for fishmeal in common carp diet (Nandeesha et al. 1990b). This may be due to the loss of growth stimulants that are probably present in the oil fraction of pupa (Tsushima and Ina 1978). Hence, the present study was designed with the twin objective of testing the efficacy of pupa oil in comparison with sardine oil in enhancing the growth rate and to evaluate the effect on growth of common carp, *Cyprinus carpio* as an additional energy source in a diet having optimal level of protein.

Materials and Methods

The experiment was conducted in triplicate in un-manured and un-aerated outdoor cement tanks of 5 x 5 x 1 m^3 without soil bed. Evaporation loss was made good by weekly replenishment with freshwater. Each tank was stocked with fish of almost uniform size (0.9 ± 0.11 g) at a density of 30 per tank. A fishmeal based diet with about 30% crude protein served as the control (Table 1). This diet enriched with either 3, 6 or 9% pupa (PO) or sardine oil (SO) formed the experimental diets. Diets were prepared using required quantity of finely ground ingredients and oil with water just adequate to prepare the moist dough (Table 1). These ingredients were cooked at 105 °C for 30 min and the cooled dough was mixed with vitamin and mineral mixture and extruded through pelletising machine to obtain pellets of 2.5 mm diameter. These pellets were dried to less than 10% moisture at 55 °C and stored in polythene bags. Fish were fed once daily at 5% body weight.

Fortnightly sampling of fish was made by collecting at least 50% of the fish using a drag net to follow growth rate. Feed quantity was adjusted based on the weight of fish recorded on the sampling day. After the experimental period of 90 days, fish from different treatments were analysed for proximate composition. Eleven trained panelists evaluated the fish quality on termination of the experiment based on various attributes like colour and gloss of skin, odour, texture and flavour of flesh (Nandeesha et al. 1988)

Fish and feed samples were analysed by the AOAC (1975) procedures, while water samples from the experimental tanks were analysed at fortnightly intervals for parameters like oxygen, carbon dioxide, alkalinity and pH following APHA (1976) procedures. Plankton samples were collected by filtering 100

Table 1. Ingredient composition of the basal diet*.

Ingredient	Percentage
Fishmeal (crude protein - 68%)	25
Groundnut oil cake (crude protein - 38%)	24
Rice bran	40
Tapioca	10
Vitamin and mineral mixture**	1

*Six diets were prepared employing this basal diet through addition of 3, 6 and 9% silkworm pupa oil or sardine oil.
**Supplevite-M, Supplied by Sarabhai Chemicals, Baroda, India.

liters of water through 60 μ bolting silk cloth and then dried at 60°C to determine the dry weight.

Specific growth rate (SGR), feed gain ratio (FGR) and protein efficiency ratio (PER) were calculated according to the following equation.

$$SGR = \frac{\text{Ln final weight - Ln initial weight}}{\text{Experimental duration (days)}} \times 100$$

$$FGR = \frac{\text{Dry weight of feed given (g)}}{\text{Wet weight gain (g)}}$$

$$PER = \frac{\text{Gain in wet weight of fish (g)}}{\text{Dry weight of protein fed (g)}}$$

The digestibility of experimental diets was determined by a short-term trial conducted in aerated glass aquaria of 77 x 38 x 38 cm. In each aquarium 10 fish of 20 ± 2 g were stocked and acclimatized to the respective diets over a period of 10 days. Chromic oxide was incorporated in the experimental diets after cooking the dough. Fish in each aquarium were fed daily with one of the experimental diets at 5% body weight at 10 hr and the left over feed was removed at 16 hr. The faecal samples were collected through siphoning the next morning at 9 hr. Water in the tank was completely changed after collection of faecal matter. Faecal samples collected over a period of 20 days from each treatment were pooled together and the digestibility was determined by employing chromic oxide (Cr_2O_3) as the marker (Furukawa and Tsukehara 1966) using the following formula.

$$\text{Apparent digestibility of nutrient} = 100\text{-}100 \; \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in diet}}$$

Data were tested for significance employing analysis of variance (ANOVA) and Duncan's (1955) multiple range test.

Results and Discussion

The water temperature ranged from 26.5-28.0 °C over the experimental period. The carbon dioxide level always remained low (0-2 ppm) while the oxygen level was always above 6 ppm (6.1-11.2 ppm); pH of water was alkaline throughout the experiment (7.0-8.2). Alkalinity (24.00-44.85 ppm) and plankton dry weight (0-32.10 mg/100 litre) remained low in all the treatments. In general, water quality parameters did not vary largely between the treatments.

The fishmeal based diet with approximately 30% protein level (Table 1) has been used for the experimental culture of carp at this Institute for many years and hence it was chosen as the reference diet. Its enrichment with different levels of oil resulted in varied proximate composition. The energy and fat levels were the highest at 9% oil incorporation (Table 2).

The growth of carp over the period of 90 days was directly related to the dietary levels of oils (Fig. 1 a & b). Although, fortnightly increase in growth and the average weight of fish recorded on termination were slightly higher for sardine oil enriched diets as compared with pupa oil diets, there was no significant variation ($P < 0.05$) at the same level of incorporation of these oils (Table 3). This clearly indicates that pupa oil is equally effective in providing additional energy to induce faster growth rate. Fatty acid analysis of pupa oil has indicated a high percentage of unsaturated fatty acids (Table 4). It was found to be particularly rich in 18 : 3 (n-3) series which are essential for many of the warm freshwater species including common carp (Takeuchi and Watanabe 1977, 1979; Tacon 1987). In addition, silkworm pupa is known to contain some attractants and growth stimulants (Tsushima and Ina 1978). Though the

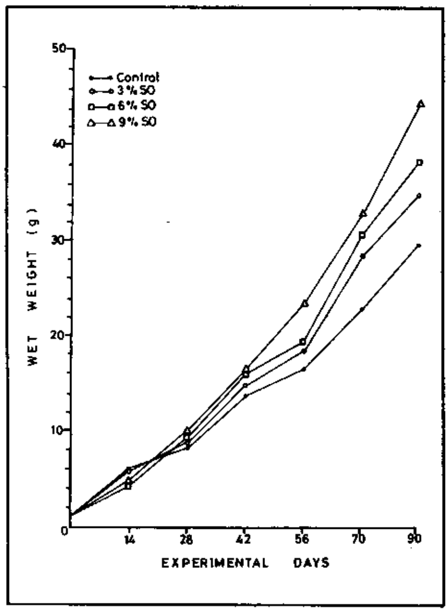

Fig. 1a. Weight gain of common carp fed sardine oil incorporated diets.

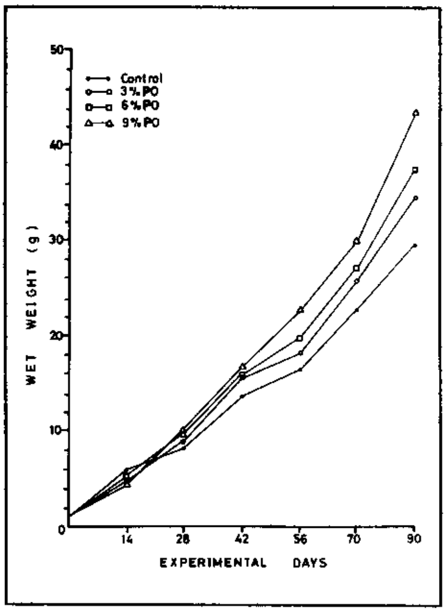

Fig. 1b. Weight gain of common carp fed pupa oil incorporated diets.

Table 2. Proximate composition (%) of formulated diets.

Treatment	Dry matter	Crude protein	Fat	Fibre	Ash	NFE	Energy (kJ g^{-1})	CP/GE
Control	95.72	29.65	7.35	12.32	15.13	31.27	14.18	2.09
3% PO	95.73	28.90	9.99	11.51	15.05	30.48	14.90	1.94
6% PO	96.48	28.00	13.26	11.62	14.03	29.57	15.81	1.77
9% PO	95.63	27.26	16.91	11.40	13.92	26.14	16.44	1.66
3% SO	95.44	28.64	10.16	12.23	14.95	29.46	14.73	1.94
6% SO	96.32	27.93	13.24	11.92	14.20	29.03	15.69	1.78
9% SO	95.37	27.20	16.10	11.46	14.80	25.81	16.07	1.69

Table 3. Growth and survival of common carp fed diets incorporated with sardine oil and pupa oil (initial weight : 0.9 ± 0.11 g; growth period 90 days).

Treatment	Average final weight (g)	Specific growth rate	Overall survival (%)*	Biomass produced (g/25 m^2)	Percent increase in biomass	Food gain ratio	Protein efficiency ratio
Control	29.47[a] (0.44)	3.84[a]	84.45	2237.70[a]	-	2.21[c]	1.53[a]
3% PO	34.46[b] (0.75)	4.01[b]	81.11	2517.00[ab]	12.48	2.18[c]	1.59[a]
6% PO	37.45[c] (0.45)	4.11[c]	85.55	2880.48[b]	28.73	1.96[b]	1.82[b]
9% PO	43.30[d] (1.32)	4.27[d]	88.89	3457.25[c]	54.50	1.79[a]	2.05[c]
3% SO	34.50[b] (0.48)	4.02[b]	81.11	2518.92[ab]	12.57	2.16[c]	1.62[a]
6% SO	38.16[c] (0.63)	4.13[c]	84.43	2897.00[b]	26.78	2.02[bc]	1.77[b]
9% SO	44.29[d] (0.39)	4.29[d]	83.34	3320.22[c]	48.40	1.88[ab]	1.96[bc]

Figures in the column with same superscript are not significantly different ($p > 0.05$).
*No significant difference between any treatments ($p > 0.05$).
Figures in parentheses indicate standard deviation.

Table 4. Fatty acid composition (%) of silkworm pupa oil and sardine oil.

Fatty acid	Pupa oil[1]	Sardine oil[2]
14 : 0	-	8.9
16 : 0	18.4	20.1
16 : 1	0.6	7.8
18 : 0	4.9	2.7
18 : 1	35.9	13.4
18 : 2 (n-6)	5.2	1.4
18 : 3 (n-6)	1.5	-
18 : 3 (n-3)	32.8	1.3
20 : 1	-	9.3
20 : 4 (n-6)	-	1.0
20 : 5 (n-3)	-	12.5
22 : 1	-	6.0
22 : 4 (n-6)	-	1.2
22 : 5 (n-3)	-	2.8
22 : 6 (n-3)	-	7.9
Unidentified	1.0	-

[1]Analysed by chromatographic technique at the Institute of Aquaculture, University of Stirling, Scotland, U.K.
[2]Tacon (1987). The nutrition and feeding of farmed fish and shrimp - a training manual. 2. Nutrient sources and composition. FAO Field Document 5.

growth of common carp observed in the present study was rather low, it is comparable with the growth recorded in earlier studies under similar conditions (Nandeesha et al. 1995; Nandeesha et al. 1998). The specific growth rate was also found to improve significantly with the increasing level of both pupa and sardine oils. Due to better survival, the total biomass production was the highest under 9% pupa oil treatment. There was almost 50% increase in biomass at 9% oil enrichment (Table 3). The trend observed in respect of feed gain ratio and protein efficiency ratio was similar to that of SGR and biomass production. Watanabe et al. (1987) reported that for common carp, it would be advisable to use high lipid (15%) with low protein (30%) levels in the diet in order to reduce nitrogen excretion and pollution of the environment without hampering fish growth. Viola et al. (1981) have also demonstrated the extra-calorific effect of different sources of oil in common carp at 5% enrichment. De Silva et al. (1991) clearly showed the possibility of using upto 18% fat in a 30% protein diet to enhance the growth rate of red tilapia. The results of the study further support the view of De Silva et al. (1991) in exploiting cheaper sources of oil as an energy source to reduce production costs.

The digestibility of pupa oil and sardine oil increased markedly with increasing levels of incorporation (Table 5). This confirms our previous work (Nandeesha et al. 1990a) in which fat digestibility increased with increasing level of pupa meal (non-defatted). Further, the fat digestibility of PO diets was significantly higher than that of the SO diets. The digestibility of fat by fish is known to be quite variable and dependent on a number of factors including the source (Appleford and Anderson 1997). Reduction in protein digestibility with increase in dietary sardine oil level has been reported in *Tor khudree* by Bazaz (1991). Also as can be seen from Table 5, the digestibility of protein is low when the fat digestibility is high. Protein digestibility was comparatively high with both oils but there was an indication of lower digestibility at the 9% PO level. On the other hand inclusion of tuna oil (Appleford and Anderson 1997) or silkworm pupa (Kitamikado et al. 1964) in the diet of common carp did not significantly affect protein digestibility.

The chemical composition of fish carcass indicated higher deposition of fat in fish treated with pupa oil as well as sardine oil, the deposition being slightly higher with the latter treatment (Table 6). Muscle/carcass fat deposition is a common phenomenon observed with higher level of fat in fish diet (Viola et al. 1981; De Silva et al. 1991; Gangadhar et al. 1997). However, in the present study the increase in fat deposition was not correlated with the dietary fat level. Hillestad et al. (1998) recorded no significant effect of high levels of dietary fat on dressed carcass fat content of Atlantic salmon. The level of deposition observed in common carp appears to be acceptable based on organoleptic quality studies (Table 7). There was no significant difference in the ash content of fish from different treatments. Except for the 3% SO diet, the protein levels were significantly higher in fish fed oil supplemented diets, indicating a protein sparing effect of the dietary fat. Similar results of protein sparing by dietary lipid has been reported in common carp (Viola et al. 1981), mahseer (Bazaz and Keshavanath 1993), rohu (Gangadhara et al. 1997) and other species as well (De Silva et al. 1991; Hillestad et al. 1998). There was an inverse relationship between protein and fat deposition in all the treatments.

The organoleptic quality of the fish showed no significant difference in respect of most of the attributes tested excepting colour and gloss of skin. In regard to this attribute, control fish was found to be better. Sardine oil incorporation at 3 and 6% resulted in lower quality of colour and gloss of skin as compared to all other treatments. Our earlier study with non-defatted silkworm

Table 5. Digestibility (%) of protein and fat in the experimental diets.

Diet	Protein	Fat
Control	82.08[d]	69.78[a]
3% PO	84.09[d]	71.37[b]
6% PO	81.22[b]	78.17[d]
9% PO	79.39[a]	82.75[e]
3% SO	84.64[d]	70.03[a]
6% SO	81.04[b]	74.00[c]
9% SO	81.72[c]	74.21[c]

Figures in same column with same superscript are not significantly different ($p>0.05$).

Table 6. Proximate composition of fish grown on oil incorporated diets**.

Treatment	Moisture	Protein	Fat	Ash*
Control	81.95[c]	13.90[a]	2.34[a]	1.10
3% PO	80.23[b]	14.09[b]	4.18[b]	1.07
6% PO	80.23[b]	14.57[c]	4.02[b]	1.07
9% PO	79.45[a]	14.14[d]	4.24[b]	1.10
3% SO	79.43[a]	13.75[a]	4.99[c]	1.17
6% SO	79.29[a]	14.17[b]	4.91[c]	1.15
9% SO	78.99[b]	14.02[b]	5.30[d]	1.17

Figures in the column with same superscript are not significantly different ($p>0.05$).
*No significant difference between treatments ($p>0.05$).
**Average of three values.

Table 7. Mean panel scores obtained for overall quality and individual attributes (The scale ranged from 1 to 4; 1 for the least and 4 for the best)

Treatment	Overall quality		Colour and gloss of skin	Colour of flesh		Texture of flesh		Flavor of flesh	Odor of flesh	
	Raw	Cooked	Raw	Raw	Cooked	Raw	Cooked	Cooked	Raw	Cooked
Control	3.36	3.14	3.55	3.27	3.09	3.64	3.27	3.09	3.00	3.09
3% PO	3.00	3.07	3.09	3.00	3.00	3.18	3.18	2.73	2.82	3.09
6% PO	3.07	2.89	3.18	2.91	2.91	3.18	2.91	2.91	3.00	2.91
9% PO	3.25	3.14	3.36	3.27	3.18	3.27	3.09	3.09	3.09	3.18
3% SO	2.77	2.66	2.82	3.00	2.91	3.09	2.91	2.64	2.73	3.00
6% SO	3.09	3.09	2.91	2.91	2.64	3.45	3.00	2.82	3.09	3.18
9% SO	3.16	2.95	3.36	3.09	3.18	3.55	2.82	3.27	3.00	2.82

pupa has shown no adverse effects on organoleptic quality of carp even at 30% level incorporation (Nandeesha et al. 1990a). Diet is known to significantly influence the quality of flesh (Spinelli 1978; Bazaz and Keshavanath 1993 ; Nandeesha et al. 1998) . In the present study, there was no odour or flavour problem even at higher level of pupa oil incorporation. However, the scores obtained in respect of various organoleptic qualities provide some indication that further increase in fat level might adversely affect the flesh quality.

The results of the present study clearly indicate the usefulness of pupa oil (1 kg = Rs.22) as an energy source whose cost is 50% less than that of sardine oil (1 kg = Rs.45). Further studies to elucidate the effect of sardine oil and pupa oil individually and in combination at different proportions and their incorporation in diets with varied protein levels would be useful. It would be more pertinent to try the usefulness of oil enrichment at lower level of protein in the diet since studies have shown the economical level of protein to be 25% for carp (De Silva et al. 1991). Further, as specific growth rate is positively correlated to optimal dietary protein content (Tacon and Cowey 1985), it may be advisable to use additional oil as an energy source in diets possessing such optimal levels in order to derive the benefit of oil in growth promotion.

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