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Replacement of cod liver oil with soybean or coconut oil in diets of larval goldfish, *Carassius auratus* L.

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ABSTRACT

A study was conducted to determine the influence of six diets, varying in lipid source and protein level on growth of goldfish (*Carasius auratus* L.) larvae during the initial period of 20 days of rearing. One day post hatching larvae were introduced into glass indoor tanks at a stocking density of 20 per 10 litre. Larvae were fed six diets in triplicate: D1 (cod liver oil, high protein), D2 (soybean oil, high protein), D3 (coconut oil, high protein), D4 (cod liver oil, low protein), D5 (soybean oil, low protein) and D6 (coconut oil, low protein) with two negative control groups (UC). After 20 days except for controls, survival was more than 75% in all the tanks. Total length of larvae was not significantly different (P>0.05) among treatments D1 (11.3 mm), D2 (11.0 mm) and D3 (11.1 mm) and among D4 (10.4 mm), D5 (10.2 mm) and D6 (10.4 mm). Based on the growth performance, it is concluded that cod liver oil could be replaced with soybean oil or coconut oil in goldfish larval diets and dietary protein requirement could be close to 30%.

Key words: fatty acids, larval feeds, *Carassius auratus*.

INTRODUCTION

Large quantities of fish meal and fish oil are consumed by aquaculture industry causing remarkable pressure to several fish stocks (Nayler *et al.*, 2000). Fish oils are rich in long chain (n-3) polyunsaturated fatty acids (PUFA), particularly 22:6(n-3) (docosohexaenoic acid, DHA) and 20:5(n-3) (eicosapentaenoic acid, EPA) which are especially required for developing neural tissues (Sargent *et al.*, 1992) and stress resistance (Sargent, 1995) respectively in fish larvae. Thus, because of their biochemical composition, fish oils are nutritionally superior to plant oils and the preferred source of lipids in larval feeds of almost all species cultured, including predominantly omnivorous groups like cyprinids (Dabrowski and Poczyczynski, 1988).

Several researchers have shown that carcass fatty acid composition of cultured fish largely reflects upon the feed. Thus from the public health view point, for food fish culture fish oils are preferred over plant oils which have more saturated and short chain fatty acids. Obviously this will not apply to ornamental fish culture as these fishes are not eaten.

Since fish oils are costly and easily get oxidised there is an incentive to replace them , partially or completely, with suitable plant oils. In ornamental fish culture, therefore, appropriate plant oils can be incorporated in feeds without compromising fish growth and survival.

Ornamental fish industry is well established in Asia (Shim, 1988) including Sri Lanka whose ornamental aquatics sector is expanding rapidly (Mee, 1993) and earns steadily increasing foreign exchange in recent times (NARA, 2001). Goldfish is one of the common and widely cultured ornamental fish (Kafuku and Ikenouse, 1983). In addition it serves as an important animal model for nutrition and other researches (Wiegand *et al.*, 1988).

Previous researches have shown that replacement of cod liver oil with canola oil did not have any adverse effect on survival and growth of goldfish larvae and juveniles (Wiegand, 1993; Pozernick and Wiegand, 1997). This study was conducted to assess the possibilities of effectively replacing cod liver oil with two cheap and commonly available plant oils in Sri Lanka, soybean oil and coconut oil, at two different protein levels in practical diets for goldfish larvae.

MATERIALS AND METHODS

Composition of experimental diets

Six experimental diets varying in lipid and protein

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sources and levels were prepared. The composition of diets is given in Table 1. These diets were prepared by mixing pulverised dry ingredients thoroughly in a blender and then adding adequate cold water, a thick paste was obtained. This was spread and dried in moderate temperature of about 40°C. The dried feeds were again ground and sieved to obtain particles in the range of 150 - 300μ.

Table 1. Composition[1] of the experimental diets.

Ingredient	D1	D2	D3	D4	D5	D6
Fish meal	50	50	50	-	-	-
Wheat flour	15	15	15	-	-	-
Soy flour	20	20	20	-	-	-
Vitamin mineral premix	5	5	5	4	4	4
Yeast	-	-	-	43	43	43
Brown rice flour	-	-	-	43	43	43
Cod liver oil	10	-	-	10	-	-
Soybean oil	-	10	-	-	10	-
Coconut oil	-	-	10	-	-	10
Crude Protein[2]	39.3	40.1	39.7	30.1	30.3	29.7
Fat (ether extract) [3]	12.6	12.8	12.4	12.8	12.8	12.5
Crude fibre	0.33	0.41	0.55	0.56	0.44	0.57
Ash[4]	2.3	2.1	2.5	2.7	2.6	2.4
Energy (cal/g) [5]	52.7	55.4	53.1	47.96	49.02	50.31

[1]Composition is given on dry matter basis.
[2]By the Kjeldahl method (N X 6.25); Tecator Kjeltec System, 1026 Distilling unit.
[3]Sohxlet extraction method.
[4]After incineration at 550°C for 16 hours.
[5]By ballistic bomb calorimetry.

Prepared diets were stored in a freezer.

Experimental set-up.

Three different experiments were carried out in glass indoor tanks each having 10 litre of clean dechlorinated tap water. Diets tested in different experiments are given in Table 2. The experiments were carried out at ambient Peradeniya conditions. Each tank was provided with separate and uniform aeration. All tanks were arranged uniformly and closely. Each experimental diet (treatment) was tested in triplicate. Treatments were arranged in a completely randomised design. Two sets of negative controls (UC) were also included in which larvae were not fed following the standard procedure of Cahu *et al.*, (1998).

Table 2. Experiments and the diets tested.

Experiment No.	Diets tested	Control
01	D1, D2, D3	UC
02	D4, D5, D6	UC
03	D1, D4	UC

Supply of fish and feeding

Goldfish larvae were obtained by semi-intensive induction of spawning in sexually mature and ripe fish as described in Vallipuram and Edirisinghe (1996). Twenty anatomically normal one day post hatching larvae were introduced into each tank and fed with the assigned experimental diet once per day, in excess. Survival rate was noted daily by removing dead larvae, if any, in each tank. Uneaten feed and faeces were also removed daily. About one third of water was exchanged daily with clean dechlorinated tap water.

Temperature, pH and dissolved oxygen content were measured by electronic pH/temperature meter and dissolved oxygen meter and ammonia content was determined by spectrophotometric method.

Mean initial total length and total wet weight were determined from fifteen randomly selected larvae from the entire pool before selection for experiments. During the course of experiments, total lengths of all the larvae in each tank were measured on 5th, 10th, 15th and 20th days. In addition on the 20th day weights of all the larvae were also measured. Specific growth rate (SGR) was determined by using the following formula.

$$SGR = \frac{[\ln(\text{mean final wet weight}) - \ln(\text{mean initial wet weight})}{\text{number of days}} X100$$

Statistical Analysis

Data were analysed by the analysis of variance procedure to determine the differences between treatments. The means were compared using Duncan's test. The statistical model used was as follows:

$Y_{ij} = \mu + Diet_i + error_{ij}$

where

Y_{ij} = total length at 5th, 10th, 15th and 20th days; wet weight at 20th day; or SGR.

μ = overall mean

$Diet_i$ = effect of i^{th} treatment (for experiment #1, i:1=D1, 2=D2, 3=D3 and 4=UC; for experiment # 2 i: 1=D4, 2=D5, 3=D6 and 4=UC; for experiment #3 i :1=D1, 2=D4 and 5=UC)

$error_{ij}$ = residual effect.

RESULTS

Temperature was the same in all tanks and range of variation was very narrow, 24.8 - 26.8 °C. Water was almost neutral with a pH range of 7.0 - 8.0. Dissolved oxygen concentration was between 6.0 - 7.0 mgl^{-1} and ammonia content was below 0.15 mg^{-1}. Survival rates at the end of the experiment were all above 75% except for the negative control group in which 100% mortality occurred before the end of the experiment. There were no sudden decreases in survival rates among fed groups (Figures 1, 2 and 3). In Experiment No. 1, on the 5th day there was significant difference ($P>0.05$) between D1, D2 and D3 (Table 3). Apart from this, there was no significant difference ($P<0.05$) in lengths, weights and SGR between fed treatments of Experiment Nos. 1 and 2 (Table 3 and Table 4). In Experiment No. 3,

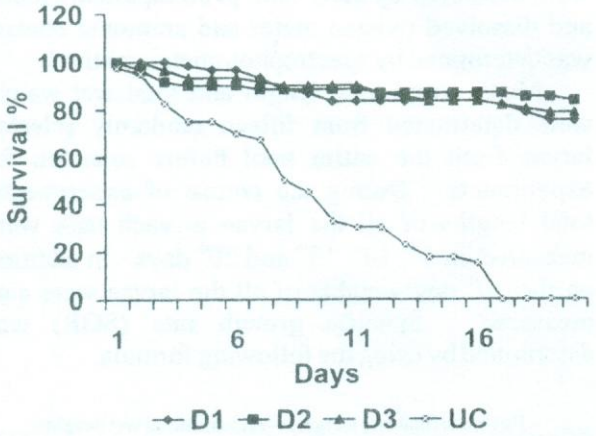

Fig. 1. Survival of goldfish larvae fed with formulated diets D1, D2 and D3.

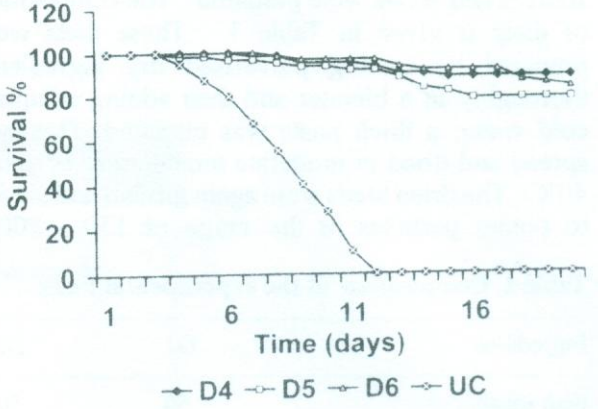

Fig. 2. Survival of goldfish larvae fed with diets D4, D5 and D6.

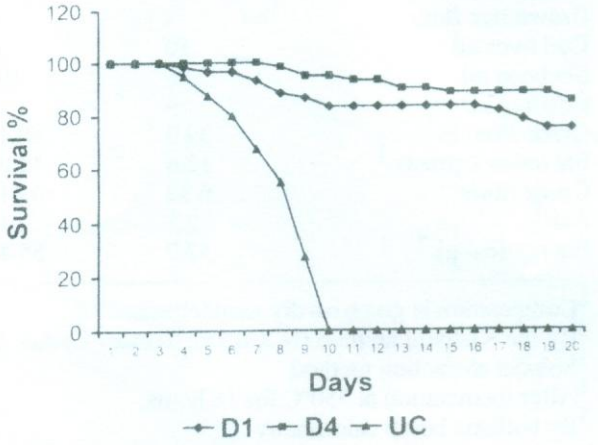

Fig. 3.Survival of goldfish larvae fed with formulated diets D1 and D4 and UC.

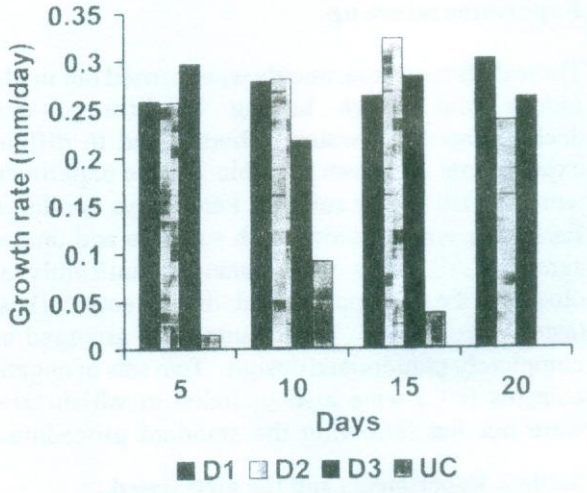

Fig. 4. Pattern of growth rates of goldfish larvae fed with formulated feeds D1, D2 and D3.

these growth parameters varied significantly ($P > 0.05$) between treatments (Table 5).

Growth rates calculated for a period of five days in terms of length did not show a clear pattern.

Table 3. Growth parameters of larvae in Experiment No. 01.

Diets	D1	D2	D3	UC
	Total length (mm) Mean ± SE			
05th day	7.1 ± 0.05[a]	7.1 ± 0.06[a]	7.3 ± 0.06b	6.5 ± 0.07[c]
10th day	8.5 ± 0.10[a]	8.2 ± 0.08[a]	8.4 ± 0.09[a]	7.0 ± 0.10[b]
15th day	9.8 ± 0.06[a]	9.8 ± 0.06[a]	9.8 ± 0.06[a]	7.3 ± 0.03[b]
20th day	11.3 ± 0.21[a]	11.0 ± 0.14[a]	11.1 ± 0.14[a]	-
	Total wet weight (mg) Mean ± SE			
20th day	14.4 ± 1.02[a]	13.3 ± 0.63[a]	13.0 ± 0.64[a]	-
SGR (% day-1)	14.4[a]	14.0[a]	13.9[a]	

[1]Within rows, means not sharing a common superscript are significantly different ($p<0.05$).

Table 4. Growth parameters of larvae in Experiment No. 02.

Diets	D1	D2	D3	UC
	Total length (mm) Mean ± SE			
05th day	6.9 ± 0.05[a]	6.8 ± 0.05[a]	6.9 ± 0.05[a]	6.4 ± 0.07[c]
10th day	7.9 ± 0.07[a]	7.8 ± 0.06[a]	7.7 ± 0.07[a]	6.7 ± 0.08[b]
15th day	9.4 ± 0.10[a]	9.4 ± 0.12[a]	9.4 ± 0.10[a]	-
20th day	10.4 ± 0.09[a]	10.2 ± 0.09[a]	10.4 ± 0.09[a]	-
	Total wet weight (mg) Mean ± SE			
20th day	10.9 ± 0.37[a]	10.4 ± 0.42[a]	10.6 ± 0.40[a]	-
SGR (% day-1)	13.0[a]	12.8[a]	12.8[a]	

[1]Within rows, means not sharing a common superscript are significantly different ($p<0.05$).

However, generally growth rates during the first five days were higher than those of last five days (Figures 4 and 5).

Table 5. Growth parameters of larvae in Experiment No. 03.

Diets	D1	D2	D3
	Total length (mm) Mean ± SE		
05th day	7.1 ± 0.05[a]	6.9 ± 0.05[b]	6.4 ± 0.07[c]
10th day	8.5 ± 0.09[a]	8.1 ± 0.48[b]	6.9 ± 0.08[c]
15th day	9.8 ± 0.06[a]	9.3 ± 0.09[b]	-
20th day	10.7 ± 0.14[a]	10.2 ± 0.09[b]	-
	Total wet weight (mg) Mean ± SE		
20th day	9.2 ± 0.38[a]	8.9 ± 0.30[a]	-
SGR (% day-1)	12.2[a]	11.9[b]	

[1]Within rows, means not sharing a common superscript are significantly different ($p<0.05$).

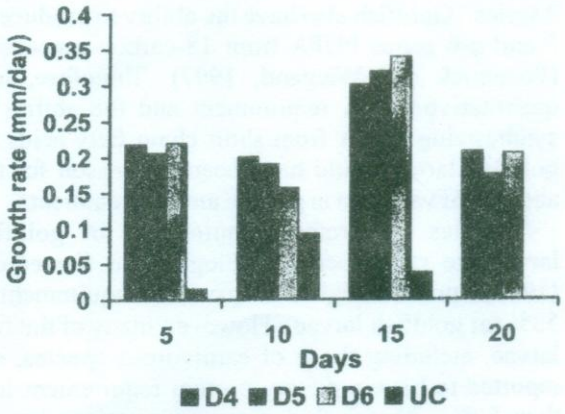

Fig. 5. Pattern of growth rates of goldfish larvae fed with formulated feeds D4, D5 and D6.

DISCUSSION

Water quality parameters observed in this study were within optimum ranges (Boyd, 1982; Alabaster and Lloyd, 1984; Hasan and Macintosh, 1986; Fiogbé and Kestemont, 1995; Kestemont, 1995).

These experiments indicated that the type of oil used in diets do not have any profound effect on larval growth, in terms of total lengths, weights and SGRs. The essential fatty acid (EFA) content varies in cod-liver oil, soybean oil and coconut oil. Cod-liver oil has about 37g of EFA/100g of total fatty acids, which mostly contain PUFAs of the groups 20:5n-3 and 22:6n-3. Soybean oil has about 60g of

EFA/100g of fatty acids and EFA in soybean oil is dominated by 18:2n-6 group (Tacon, 1990). Coconut oil has very low, about 3g of EFA/100g of fatty acids, which largely contains 18:2n-6 group (Tacon, 1990). Despite this variation among three oils, larval growths did not vary significantly. Similar results were reported by Pozemick and Wiegand (1997) when goldfish larvae and juveniles were fed with 10% cod-liver oil or 10% canola oil. Growth of common carp, *Cyprinus carpio* L., larvae did not vary when they were fed with tryglyceride triolin (10%) or coconut oil (10%) (Fontagné *et al.*, 1999). Average weight gain of goldfish fed with diets containing 4.0% cod-liver oil or 4.0% soybean oil for a period of six weeks did not show any significant difference (Lochmann and Brown, 1997). The absence of variability can be attributed to the possible low n-3 fatty acid requirement in goldfish. Though PUFA requirement in goldfish is not precisely known, it is assumed that the requirements are similar to those of common carp (Lochmann and Brown, 1997). Studies with carp larvae (Geurden *et al.*, 1995) indicated that trace amounts, 0.05-0.1% of the n-3 fatty acids in the dry diet satisfied the PUFA requirements.

Furthermore, freshwater fish mainly require n-6 series of EFA than n-3 series (Tacon, 1990). Also with the exception of strict carnivorous fish species, fish are able to chain elongate and further desaturate 18:2 n-6 or 18:3 n-6 to 20:4 n-6 or 20:5 n-3 or 22:6 n-3 series. Goldfish also have the ability to produce n-3 and n-6 series PUFA from 18-carbon precursors (Pozemick and Wiegand, 1997). Therefore, low quantitative PUFA requirement and the ability of synthesizing PUFA from short chain fatty acids by goldfish larvae could have been the reason for the absence of variation in growth among treatments.

Studies on protein requirement of goldfish larvae are rather scanty. Fiogbé and Kestemont (1995) reported a high dietary protein requirement of 53% for goldfish larvae. However, many of the fish larvae, including those of carnivorous species, are reported to have a dietary protein requirement less than 50%. This includes common carp and grass carp (*Ctenopharyngdon idella* L.), which are closely related to goldfish taxonomically and the former (common carp) having food habits similar to that of goldfish. Common carp and grass carp require 34-38% and 41-43% dietary protein respectively for optimal growth (Tacon, 1990). Thus the actual dietary protein requirement of goldfish may be much lesser than 53%. SGR of goldfish larvae fed with 53% protein diet was 12.7 %day^{-1} (Fiogbé and Kestemont, 1995). However in this study diet A4 that had dietary protein level of 30.1% resulted in a SGR of 13.0%day^{-1}. This implies that the dietary protein requirement of goldfish larvae is less than 53% and could be close to 30%.

The general pattern of growth rate observed in this study is in agreement with the accepted hypothesis that larval growth rate is highest during initial stages and tends to decrease as the larvae grow. This is because rapid growth is of supreme importance to young fish larvae, as mortality due to predation declines rapidly with increasing size of the larvae (Pedersen, 1997).

The results of this study showed that expensive cod liver oil can be completely replaced with cheaper soybean or coconut oils in goldfish larval feeds. Further research must be conducted in estimating a more precise protein requirement of goldfish larvae under Sri Lankan conditions.

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