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Cost-benefit analysis of protein ingredients in the maturation diets of goldfish, *Carassius auratus* (Linnaeus, 1758)

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Abstract
A comprehensive trial was conducted with three different protein sources such as Spirulina, earthworm and shrimp head meal for their cost-benefit analysis in the growth and maturation of goldfish was conducted. Troughs (45 *l* cap) were used for stocking and maintaining goldfish in replicates. Each ingredient was supplemented with two different concentrations (10% and 15%) and control diet was also made without any special ingredients. Sampling on biogrowth and gonad development parameters was done once in 10 days and 30 days, respectively. The highest feed conversion (75.89%) was observed with the diet containing shrimp head meal at 15% level (T_{31}) and highest GSI (31.54%) was observed in fishes fed with diet containing Spirulina at 10% level (T_{12}). This was found positive when cost-benefit analysis was done. Statistical analysis of data also revealed significant difference at 99% level in the GSI of the fishes fed with different diets and control feed.

Keywords: Low cost feed ingredients, biogrowth, gonad development, goldfish

Introduction

Goldfish is one of the most important ornamental fishes for trade in international market [20] with high economic value in Japan [21]. The breeding and production of young ones remain as an attractive enterprise. Maturation of adults and use of suitable maturation diets are important for the development of gonads.

The dietary requirements of broodstock are different from those of rapidly growing juvenile fishes as stated by Leboulanger (1977). The role of nutrients and supplementary feeds on regulating the reproductive physiology of broodstock is well documented by Pavlov *et al*. (2004). According to the available reports, it is understood that nutrient requirements vary depending on species, size, developmental status, sex, and gonad and maturation process [30]. Influence of feed on growth, gonad maturation and reproduction in ornamental fishes was also well highlighted [15]. Reduction in feeding rate caused an inhibition of gonadal maturation in several fish species including goldfish [28]. Also it was observed that gonadal maturation, fecundity, fertilization, embryo development and quality of larvae are greatly affected by the absence of certain nutrients in the feed [30]. Therefore, Optimum nutritional support is essential for fish to get maturation in confined waters.

In formulated feeds, selection of ingredients is a major task to arrive at an economically viable to feed for application in the field. Fish meal remains as a major source of protein. Excessive use of such protein rich ingredients leads to water pollution which may lead to fish death. Further availability of fish meal is also in critical situation due to stagnation in capture fisheries and increased price [11, 23]. Therefore, there is a need to select an alternative protein source for preparing commercial feeds for ornamental fishes.

There are two types of alternative protein sources for fish meal such as plant origin and animal origin. Spirulina is a plant protein source which has no anti nutritional factors and cellulose in the cell wall. It has mucoprotein which can enhance the digestibility of fishes consequently increases growth of fishes.

Earthworm is a cheap animal protein and can effectively compensate the protein requirements of fishes [12]. Many researchers have reported that earthworm is a possible alternative additive and also a fish meal replacer in aqua feeds [9]. Earthworms can also provide a substantial nutrition to the animals consuming them [22].

In shrimp processing plants, the cephalothorax portion of shrimp is considered as a waste. But, it is as an excellent animal protein additive in aquaculture due to its rich protein, cholesterol, free fatty acids and mineral contents [5].

These three non-conventional feed additives were taken for the present study and cost-benefit of the ingredients was calculated in the light of growth and maturation.

Materials and Methods

Juveniles of goldfish, *Carassius auratus* with body weight ranging from 3 to 5 g were purchased from an ornamental fish breeding farm near Thoothukudi. They acclimatized for a period of 15 days with control feed and then individuals with an average body weight of 5.79±0.91g were stocked at a density of 4 numbers per trough in replicates. They were fed with experimental diets to 4% of their body weight per day with experimental diets [17]. The feeding frequency of fishes was three times a day viz., 9 am, 1 pm and 5 pm. The body weight was recorded once in 10 days for 60 days and the quantity of feed was adjusted at each sampling based on the weight of the fishes. Once in 30 days, one fish from each treatment was sacrificed to harvest the gonad for histological sectioning and observation.

Experimental feed preparation

The experimental feeds were prepared based on the procedure of Cho *et al*. (1985). Spirulina flakes were purchased from a Spirulina production farm (Rs. 400/Kg). The Spirulina flakes were ground well and sieved to get uniform size particles for the feed preparation [18].

Table 1: Feed formulae and the list of ingredients used in experimental diets

Sl. No	Ingredients	T_{11}	T_{12}	T_{21}	T_{22}	T_{31}	T_{32}	C
1	Ground nut oil cake	20	20	20	20	20	20	20
2	Rice bran	35	40	40	35	40	35	50
3	Wheat bran	5	5	5	5	5	5	5
4	Maize flour	7.5	7.5	7.5	7.5	7.5	7.5	7.5
5	Salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	Vitamin & mineral mix	0.1	0.1	0.1	0.1	0.1	0.1	0.1
7	Fish meal	15	15	15	15	15	15	15
8	Fish oil	2	2	2	2	2	2	2
9	Yeast	0.1	0.1	0.1	0.1	0.1	0.1	0.1
10	Vitamin E	0.2	0.2	0.2	0.2	0.2	0.2	0.2
11	Spirulina meal	15	10	0	0	0	0	0
12	Earthworm meal	0	0	15	10	0	0	0
13	Shrimp head meal	0	0	0	0	15	10	0
	Total	100	100	100	100	100	100	100

Fresh earthworm was procured from KVK Vermifarm (Rs. 1600/Kg). The earthworms were washed with running water to remove the adhering soil and other matters. The cleaned earthworms were weighed and they were depurated in freshwater for 30 minutes to clean their intestinal load from the gut [2]. The live earthworms were killed in an ethical way by blanching in moderate salt water containing 10g/*l* for 30 minutes. The dead earthworms were once again weighed and dried in air. Then they were ground in a dry grinder to powder [10] and sieved for preparation of earthworm meal.

Table 2: Cost details of the different ingredients used in the experiment

Si. No	Experimental additive included & Treatment Code	Inclusion level of additive (%)	Cost of the additives (Rs./kg)	Cost in the feed (Rs./kg)
1	Control (C)	0	0	0
2	Spirulina (T_{11})	15	Rs. 400	Rs. 60/-
3	Spirulina (T_{12})	10	Rs. 400	Rs. 40/-
4	Earthworm meal (T_{21})	15	Rs. 1600	Rs. 240/-
5	Earthworm meal (T_{22})	10	Rs. 1600	Rs. 160/-
6	Shrimp head meal (T_{31})	15	Rs. 120	Rs. 18/-
7	Shrimp head meal (T_{32})	10	Rs. 120	Rs. 12/-

Shrimp head wastes were collected from processing plant (Rs. 120/Kg) and dried under sun after thorough washing. The unwanted shrimp appendages and detritus were removed and the dried shrimp head wastes were then ground in a dry grinder to make the powder and sieved to get uniform sized particles [14].

Proximate composition such as moisture, crude protein, crude fibre, ether extract, total ash content and gross energy of experimental diets were determined by using standard analytical methods [4]. The proximate compositions of experimental feeds are given in Table 3. The feeds were isoproteinecious (32.69±1.38) and isocalorific (3948±47.14).

Table 3: Estimated proximate composition of experimental feeds

Proximate composition	T_{11}	T_{12}	T_{21}	T_{22}	T_{31}	T_{32}	C	Mean±SD
Crude protein (%)	32.76	32.88	32.00	31.82	32.22	31.93	32.43	32.72±1.38
Crude fibre (%)	4.25	4.69	3.66	3.76	11.26	9.99	6.28	6.27±3.12
Ether Extract (%)	5.52	5.75	8.25	8.87	6.48	5.16	4.32	6.33±3.12
Gross energy (kcal/kg)	3990	3957	3436	3485	4011	3924	3897	3617±579

Estimation of bio-growth parameters

Bio-growth parameters such as growth, survival, feed consumption rate, weight gain (g), weight gain percentage and specific growth rate were estimated based on the body weight. The mean weight gain was calculated as the difference between initial and final weight of fishes in each trough [26].

Sample fishes were sacrificed to observe Gonado Somatic Index before starting the experiment. The further development of gonad was observed at 30th and 60th day of the experiment. The GSI was calculated using the formula given by Billard *et al*. (1993). The collected gonads were preserved in 10% formalin [13]. The preserved gonads were then mounted in slides at a pathology lab. The histological sections of gonad were observed under microscope to study the development of gonads at 30 days interval period during the experiment.

The collected data and the estimated parameters were subjected to 'F' test for finding out statistical significance. The growth parameters were detailed using Regression analysis and compared using ANOVA.

Bio-growth parameters such as growth, survival, food consumption rate/day, weight gain (g), weight gain percentage and specific growth rate were estimated based on the data recorded [31] which are given below:

Feed consumption / day (g) = feed given on dry matter/day-faecal matter excreted as dry matter /day (g)

Feed consumption rate / day = Total feed consumed / No. of days

Weight Gain (g) = Final wet weight (W_t) -Initial wet weight (W_0) (g)

$$\text{Mean weight gain (\%)} = \frac{\text{Final weight } (W_t) - \text{Initial weight } (W_0)}{\text{Initial weight } (W_0)} \times 100$$

$$\text{Specific Growth Rate (SGR \%)} = \frac{\ln W_t - \ln W_0}{T} \times 100$$

Where,

W_0 – initial wet weight of fishes (g); W_t – final wet weight of fishes (g); T – days of culture of fishes

$$\text{Gonado Somatic Index (GSI)} = \frac{\text{Weight of gonad (g)}}{\text{Body weight of fish (g)}} \times 100$$

Results

Analysis of cost of feeds when incorporated with different feed additives reveals that there was a great influence on the cost due to the inclusion of the selected feed additives. As it can be observed, the feed additives did not have uniform cost and therefore there was a variation of cost of the feeds (Table 2).

It has been identified from Table 4. that the diet contained earthworm meal at 15% showed highest cost of feed, which was 246% higher than control feed cost followed by 10% earthworm meal containing diet (176%).

Table 4: Cost-benefit analysis of different diets with regard to the control diet.

S. No	Treatment	C vs T_{11}	C vs T_{12}	C vs T_{21}	C vs T_{22}	C vs T_{31}	C vs T_{32}
1	Cost-benefit on feed conversion rate (%)	+23(T_{11})	+24(T_{12})	+31(T_{21})	+64(T_{22})	**+171(T_{31})**	+9(T_{32})
2	Cost-benefit on cost of feed production (%)	+63(T_{11})	+42(T_{12})	+246(T_{21})	+176(T_{22})	+16(T_{31})	+11(T_{32})

Besides the control feed, the next lower cost was found with diet containing shrimp head meal at 15% followed by 10% (16% and 11% higher than the control feed respectively). However, the feed conversion rate of fishes fed with diet containing shrimp head meal at 15% and 10% had 171% and 9% higher than the control feed, respectively indicating a wider difference in the effect of the feed additives.

In the present experiment, the fishes fed with control diet showed the lowest GSI (4.22%) indicating the effect of special ingredients incorporated diets on the maturation also (Table 5). As seen in Table 5, average cost of feed was observed with diets containing Spirulina at 10% (Rs.127/-) and 15% (Rs.146/-) which had 1.5 times higher feed cost than control diet (Rs.89/-). But, the highest GSI was observed in fishes fed with diet containing Spiriulina at 10% (31.54%), which was seven times higher GSI than control fishes followed by Spirulina at 15% (23.75%), which was five times higher GSI than control fishes. The difference of GSI between the control group and the experimental diets fed groups was statistically significant at 99% level.

Table 5: Observations on the Gonado Somatic Indices (GSI) of the *C. auratus* when fed with feeds incorporated with three different feed additives at two different inclusion levels

SI. No	Experimental additive included & Code	Inclusion level of additive (%)	Cost in the feed (Rs./kg)	MeanGSI$\pm$SD (%)
1	Control (C)	0	89.545/-	4.22±0.34
2	Spirulina (T_{11})	15	146.545/-	23.75±1.24*
3	Spirulina (T_{12})	10	127.545/-	31.54±5.06*
4	Earthworm meal (T_{21})	15	326.545/-	13.64±2.14*
5	Earthworm meal (T_{22})	10	247.545/-	12.69±2.26*
6	Shrimp head meal (T_{31})	15	104.545/-	8.04±0.35*
7	Shrimp head meal (T_{32})	10	99.545/-	9.97±0.11*

*$P < 0.01$

Discussion

As seen in Fig. 1, the cost of control feed was the least when compared to other experimental diets, which means that the special feed ingredients were greatly influenced the cost of feeds in the experiment. As it is evident from fig. 2, the lowest feed conversion was observed in control group fishes while all the experimental feeds showed higher feed conversion than the control feed, which signals the positive influence of special feed ingredients on the feed conversion rate of fishes.

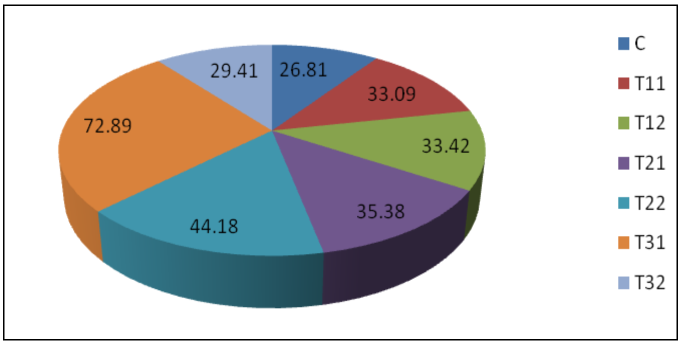

Fig 2: Flesh conversion (%) using different feeds in the experiment

The diet containing shrimp head meal at 15% is adjudged as the cost effective feed in the present experiment. This is in accordance with the studies of Akiyama *et al*. (1991) on the cost effective growth in aquatic animals. They described that shrimp head meal is low cost meal which contains higher quantity of lysine and arginine and the diet with 15% shrimp head meal had the ratio of lysine and arginine closer to the recommended ratio for growth of aquatic animals. It probably might have improved the amino acid profile in the diets and enhanced the growth in animals.

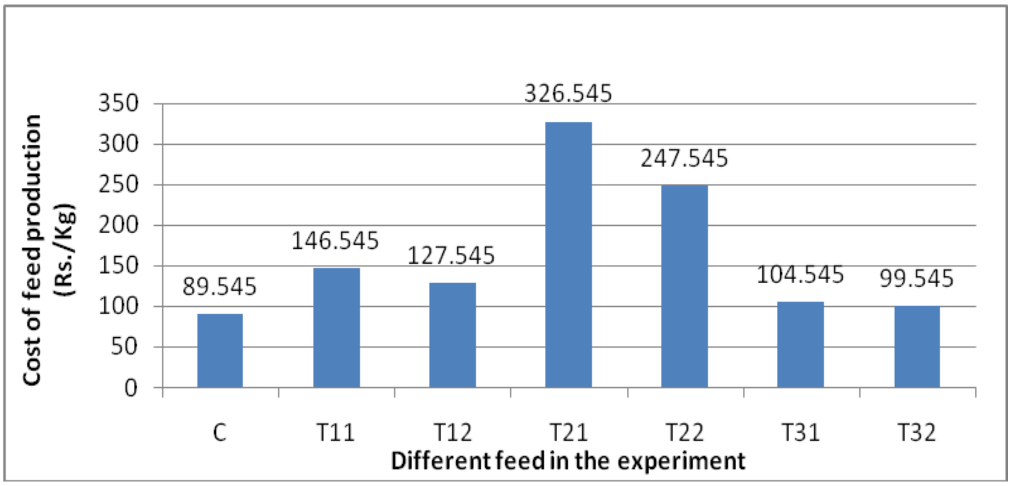

Fig 1: Cost of production of different feeds in the experiment

They also reported that less than 15% shrimp head meal contained diet reduced the protein availability to fishes consequently reduced the growth of fishes. The same result was observed in the studies done by Barbarito *et al*. (2009), who reported that 10% shrimp head meal containing diet did not give better growth for juveniles of *Litopenaeus schmitti*. However, high inclusion level of shrimp head meal in the fish feed would increase the cost of feed and forced to reduce the digestibility in fishes as observed by Nwanna (2003). This was reported due to the presence of exoskeleton, chitin and ash content. It was also confirmed by Barbarito *et al*. (2009) who reported that inclusion of shrimp head meal greater than 25% in feed increased the cost of feed and reduced growth through low feed utilization in *L. Schmitti* juveniles. Therefore, care should be taken in the inclusion of shrimp head meal in fish diets.

Olele *et al*. (2014) pointed out the highest cost of feed due to incorporation of earthworm meal when he worked with fingerlings fed with diet containing fish meal replaced with earthworm meal at 100% level. However, the earthworm meal incorporation did not high give high growth as observed by Zakaria *et al*. (2012). They reported reduced growth of fishes when Catfish was fed with feed containing 15% earthworm meal and opined that it was due to the reduced protein availability to fishes when Catfish was fed with feed containing 15% earthworm meal. It is falling in line with the observed in *Heterobranchus longilifilis* fingerlings, when they were fed with feed containing earthworm meal at 15 - 25% level, which increased the cost of feed production [29].

James *et al*. (2006), who examined the cost effective maturation of fishes observed four times greater gonad weight in Swordtail when fed with 8% Spirulina diet at average feed cost. This attributed to due to greater availability of protein and gonad stimulatory substances from Spirulina. When Spirulina concentration is further increased in feeds in the present study, it was found to increasing feed cost and also decrease feeding efficiency consequently reducing growth and gonad development. The same result was also reported by Allen (2016) in Nile Tilapia fed with diet containing Spirulina at 15% which caused better growth and maturation in fishes.

Though, diet containing shrimp head meal at 15% had low cost of production (Rs.104/-) but the experimental fishes fed with the same diet produced only very marginal two times increment of GSI than the control feed (8.04%). So, it is assumed that the diet containing shrimp head meal did not have suitable ingredients for maturation of goldfishes. The same result was reported by the author Akiyama *et al*. (1991) who, reported that shrimp head meal at 15% containing diet have suitable nutritional quality only to improve the growth in juveniles of *Litopenaeus schmitti* at lesser cost and it has no influence on maturation of animals.

In the present experiment, the highest cost of feed production (Rs. 326.55/ kg) was observed in diet containing earthworm meal at 15%, which was 246% higher than the control feed, when fishes fed with the feed produced only three times higher GSI (13.64%) than control feed. Hence, the diet containing Spirulina at 10% level is adjudged suitable low cost of feed for setting higher maturation in goldfish.

Conclusion

From the present study, based on the observation of cost and benefits, the highest feed conversion (72.89%) was observed in fishes fed with diet (T_{31}) containing shrimp head meal at 15% level and the highest GSI (31.54%) was observed in fishes fed with diet containing Spirulina at 10% level. So, it is concluded that the diet containing shrimp head meal at 15% is a low cost and high beneficial feed for growth and diet containing Spirulina at 10% is a low cost and high beneficial feed for maturation of goldfish.

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