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Effect of Squillameal on the Growth and Nutritive Value of *Labeo rohita* (Ham)

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Abstract

It has been well established that animal protein performs better than plant protein in the growth and nutritive value of cultivable fish. By far the most suitable animal protein for incorporation into fish diets is fish meal. Fish meal is almost always made from marine fish, and can be based on the by-catches or a product of a specific fishery (De Silva and Anderson, 1995). Squilla species, a marine stomatopod generally called as mantis shrimp, is found in abundance along the Indian coast. The fine powder obtained from grinding the whole body of sun dried squilla species is known as squillameal, which has an excellent nutritive value. Taking this into consideration, it has been proposed to evaluate the suitability of incorporation of squilla meal as animal protein, in the traditional feed (rice bran and groundnut oil cake mixture) of *Labeo rohita*. For this purpose experiments have been conducted on the fingerlings (of an average weight of 5.33±0.13g) of *L. rohita*, for 90 days in the laboratory with 35% protein level traditional feed as control diet and 35%and 40% protein level feeds, formulated with squilla meal, as test diets. All the ingredients of these diets have been sieved, steam cooked after through mixing with water, pelletized with the help of a pellet mill and then dried in a hot air oven with blower at about 50^{o} C. The dry pellets of these diets have been used to feed the experimental fingerlings of *L. rohita* to study the Absolute growth, Specific growth rate (S.G.R), Food conversion ratio (F.C.R), Protein efficiency ratio (P.E.R), and Apparent protein digestibility (A.P.D). The observations revealed that the performance of both the test diets formulated with squilla meal**,** is far superior to that of the control diet. However, from among the two formulated diets, the one with 35% of animal protein performed better in every respect. Proximate analyses of all the three diets and fish flesh have been carried out to evaluate the relative performance of the two animal protein incorporated artificial diets. The details are dealt in the present paper.

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Introduction

Fresh water aquaculture in India is mainly carp- based and accounts for about 89% of total aquaculture production. Over the last one and half decades, fish farming has been fast developing from the traditional extensive system to the semi-intensive and intensive culture systems, by increasing the stocking density. As the density exceeds the natural carrying capacity, to achieve optimum growth and increased production, the dependence shifts from natural food to nutritionally favorable protein rich artificial food. The quality and quantity of dietary protein influence growth, provided other physiological requirements for growth are fulfilled. In general the dietary protein requirement of the fish ranges between 35% and 55% or an equivalent of 47% - 75% of the gross energy content of the diet in the form of protein (Tacon and Cowey 1985). Since protein is the most expensive dietary constituent of feed and as such feed accounts for the largest cost in fish culture, there is an increasing attempt to develop cost effective feeds with most economical level of dietary protein for profitable fish culture. By far the most suitable animal product for incorporation in to fish diets is the fish meal. Many experiments with catfish and carp have shown that fish meal (animal protein) has a special importance in the diet and the replacement of fish meal by a vegetable protein source such as soybean oil meal, resulted in decreased growth rate of the fish (De Silva and Anderson, 1995).

The objective of the present study was to formulate and evaluate a suitable artificial feed, by incorporating a cost effective animal protein for *Labeo rohita* (Cyprinidae) – an indigenous major carp, cultured widely in the state of Andhra Pradesh. Accordingly squilla meal has been selected as the source of animal protein to find out its effect at different dietary protein levels, on the growth and nutritive value of the *Labeo rohita*, in comparison to the traditional diet of rice bran and ground nut oil cake mixture.

Squilla, a marine stomatopod, forms a major portion of the trash fish landings in Coastal Andhra Pradesh. It is often observed that this precious animal protein is mostly getting wasted without being used either for direct human consumption or for production of fishmeal. Visakhapatnam has a very big fishing harbour, where many mechanized boats operate and their catch consists of considerable quantity of trash fish including the squilla. However, there is no available data regarding squilla landings in Andhra Pradesh coast, the current estimated stomatopod landings in India

during the years 2002 and 2003 are 48,551 and 37,341 tonnes respectively (CMFRI, 2004). As this highly valuable animal (crustacean) protein is abundantly available, it has been proposed to formulate some cost effective feeds that could work well in the fin fish culture in and around Visakhapatnam.

Materials and methods

Experimental Diets

Specimens of squilla species, collected from the fishing harbour of Visakhapatnam, were thoroughly washed, sun dried and ground to fine powder. Rice bran and groundnut oil cake were procured from the local markets of Visakhapatnam. All the ingredients were sieved to obtain a fine powder and kept in an airtight container carefully. The proximate biochemical composition of each of the above mentioned ingredients was analyzed and by using them, three diets namely one control diet (D1) and two test diets (D2 and D3) were prepared following the Square method. Since the experiment was designed to evaluate the effect of squilla meal, as it is, hence there is no addition of vitamin and mineral mixture in the formulation of diets. The ingredients of each diet were made to a dough by adding water and it was cooked in a steam pressure cooker as followed by Jayaram and Shetty (1981). After cooking the dough was passed through a mechanical pelletiser, to obtain pellets of 2 mm diameter, which were dried in a hot air oven with blower at 50^{o}C for 24 hours. These pellets were stored in an airtight container for further use.

Experimental set up

The experiments were carried out in fiberglass tanks of 80 L capacities for a period of 90 days. Ground water stocked for about 24 hours was used to fill the tanks. The physicochemical characteristics of water were examined following the standard procedures given in APHA (1980). Twelve fingerlings of rohu, of initial weight 5.33 g ± 0.13 (mean ± S.D) were stocked in each tank and aerated with mechanical aerators to avoid oxygen depletion.

Table 1. Proximate composition (%) of ingredients used in feed formulations (% dry weight)

PARAMETERS	RICE BRAN	GOC	SQUILLA POWDER	TAPIOCA FLOUR
Moisture	11.20	6.57	1.80	7.58
Crude Protein (N × 6.25)	14.68	44.27	49.50	3.64
Carbohydrate	46.21	31.59	5.29	83.6
Lipid	18.60	10.00	16.40	4.40
Ash	9.31	7.57	27.00	0.78
Chitin	*	*	11.68	*

* Not determined

Table 2. Ingredient proportion and proximate composition of experimental feeds (% dry weight)

INGREDIENTS	D1 (35%)	D2 (35%)	D3 (40%)
Rice Bran	20	28	17
Groundnut Oil cake	72	- -	- -
Squilla meal	- -	62	75
Tapioca flour	8	10	8
PROXIMATE COMPOSITION (%)			
Moisture	3.26	2.28	3.10
Protein	35.48	35.56	40.00
Carbohydrate	39.96	21.62	18.20
Lipid	13.40	13.60	18.80
Ash	8.38	17.46	19.90

Experiments were conducted in two replicates, in indoor laboratory conditions. Water exchange was done every day in the morning hours. There is no natural food presented in the experimental tanks. According to Carlos (1988) there is no relationship was found between the growth parameters and feeding frequency. Windell (1976), in his feeding frequency study on rainbow trout, did not find any influence on the growth when feeding frequency was increased above once per day. Hence the fish were fed with respective diets at 5% of their body weight, once in a day in the morning hours. On every 10^{th} day the fish were weighed and the quantity of ration was adjusted accordingly. The left over feed was collected at 6.00 pm daily, separately from each tank and weighed after drying in an oven to determine the feed consumption. Faecal matter was collected at 8.00 a.m. every day by siphoning, prior to feeding and kept separately for each group of fish after drying, for proximate analysis to determine the apparent protein digestibility. At the termination of the experiment, 6–8 fish from each

treatment were sacrificed and analyzed for proximate composition of the skeletal muscle.

Biochemical composition

Proximate composition of all the ingredients, diets and fish muscle was determined by following the procedures of AOAC (1980) as follows; semi-micro Kjeldahl method for protein, Dubois et al. (1956) method for carbohydrates, and Folch et al. (1957) method for lipids. Apparent protein digestibility was determined by following the method of De Silva and Anderson (1995), using acid insoluble ash (AIA) as an internal marker.

Data analysis

The performance of the diets, in terms of weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and apparent protein digestibility (APD) was determined by using the following formulae. The data were subjected to one-way analysis of variance (ANOVA) on a personal computer. Mean differences between treatments were tested for significance at $P < 0.05$ and comparison was made by Duncan's multiple range multiple F-test (Duncan 1955). Since the faecal matter of replicate experiments were pooled together, it is not possible to subject the apparent protein digestibility data to ANOVA.

$$\text{Weight gain}(\%) = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \text{ x } 100$$

$$\text{Specific growth rate (SGR; \%day}^{-1}) = \frac{l_n \text{ Final body weight} - l_n \text{ Initial body weight}}{\text{Experiment period in days}} \text{ x } 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Apparent nutrient digestibility with AIA as marker (%)

$$= 100 - \left[100 \text{ x } \frac{\%\text{ marker in diet}}{\%\text{ marker in faeces}} \text{ x } \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in diet}} \right]$$

Results

The temperature, pH, dissolved oxygen content and alkalinity of the water in the experimental tanks ranged from 28 – 32°c; 7.4-8.3; 5.6-7.3 mg l^{-1} and 208 to 224 mg l^{-1} respectively. The growth performance and feed utilization efficiency of *L. rohita* fingerlings fed with different diets are summarized in table 3. The net weight gain, live weight gain (%) and SGR (% day^{-1}) were more in the fish fed with diet D_2 (35% total protein) and diet D_3 (40% total protein) than in those fed with control diet D_1, which contains 35% protein of plant source. Between the test diets D_2 and D_3, diet D_2 with 35% animal protein showed better performance in terms of FCR and PER also. The apparent protein digestibility of diets D_2 and D_3 was found better than that of the control diet D_1. The protein and lipid content in the muscle of the experimental fish were considerably higher in the finger lings fed with the test diets. In between the two animal protein diets, the fish fed with diet D_2 showed higher amount of protein and lipid in the muscle compared to those fed with D_3. Significant difference in performance of the diet D_1 when compared to D_2 and D_3 was observed in terms of the protein content of the fish. The lipid content in the muscle of

the experimental fish was significantly high in the fish fed with the D_2 than the control diet D_1. The difference in the performance of diets D_1 and D_3 was not significant when compared to that of D_1 and D_2. The survival is > 83% in all groups.

Analysis of variance (ANOVA) indicates that the growth parameters and feed utilization efficiency were significantly different for the three diets ($p < 0.05$). The net weight gain, live weight gain (%), SGR, FCR and PER were the best in fish fed with diet D_2. A comparative analysis of the performance of the diets D_1 (control diet), D_2 and D_3, by the multiple range test indicates that the net weight gain, live weight gain (%), SGR, FCR, and PER differed significantly between the fish fed with the diet D_1 (control diet) and fish fed with the diets D_2 and D_3. However, between the fish fed with the diets D_2 and D_3, no significant differences were observed excepting in the protein efficiency ratio.

Discussion

In the present study, the differences observed in thee performance of the control and test diets were quite significant. Both the test Diets D_2 and D_3 with animal protein, performed better than the control diet D_1, which is of only plant protein. According to Cowey et al. (1972); Satia (1974) and Cho et al. (1976), dietary protein plays a dominant role in fish growth. Akiyama et al. (1981) and Winfree and Stickney (1984) established that when protein level in the diet increases, gain in body weight becomes pronounced. In the present experiment both the squilla meal incorporated test diets performed better than that of the traditional rice bran and oil cake mixture diet (control diet) and this is in agreement with the observations of De Silva and Anderson (1995), who stated that animal by-product meal, particularly fish meal appears to contain unidentified growth factors. Between the two test diets the diet with 35% protein (D_2) performed better than the diet with 40% protein (D_3), suggesting that protein beyond an optimum level is poorly utilized for growth. Cowey et al. (1981) demonstrated that gluconeogenic enzyme increased in rainbow trout when fed on high dietary protein indicating that with high protein diets, protein is increasingly catabolised for liberation of energy. Singh and Dhawan (1996) made similar observations elsewhere.

Table 3. Growth performance and feed utilization efficiency of *Labeo rohita* fingerlings fed with experimental diets

Parameters	D1	D2	D3
Avg. Initial weight (g)	5.33 ± 0.11	5.33 ± 0.13	5.33 ± 0.15
Avg. Weight gain (g)	4.83 ± 0.79^{a}	9.09 ± 0.97^{b}	7.29 ± 0.96^{b}
Avg. Gain in length (cm)	1.13 ± 0.35^{a}	2.03 ± 0.29^{b}	1.42 ± 0.37^{ab}
Avg. Live weight gain (%)	90.71± 14.9^{a}	170.68 ± 18.17^{b}	140.86 ± 21.62^{b}
Specific Growth Rate (SGR;% day^{-1})	0.707 ±0.08^{a}	1.106 ± 0.07^{b}	0.955 ± 0.08^{b}
Feed intake (g fish^{-1} day^{-1})	0.24 ± 0.02 ^{a}	0.26 ± 0.02 ^{a}	0.23 ± 0.01 ^{a}
Feed Conversion Ratio (FCR)	4.42 ± 0.72^{a}	2.54 ± 0.27^{b}	2.89 ± 0.38^{ab}
Protein Efficiency Ratio (PER)	0.62 ± 0.5^{a}	1.12 ± 0.07^{b}	0.84 ± 0.06^{c}
Apparent Protein Digestibility (APD)[1]	86.35	95.75	95.80
Survival (%)	83.34 ± 8.3^{a}	87.5 ± 4.17^{a}	87.5 ± 4.17^{a}

The results are means ± S.D. and the figures with same superscripts in the same row are not significantly different ($P < 0.05$).

[1]Statistical analysis was not possible as determinations were performed on pooled samples

Protein efficiency ratio (PER) decreased with increased protein level in the diet. Higher protein content was observed in the muscle of fish fed with 35% protein level diet – D_2, than in those fed with a higher protein (40%) level diet – D_3. The excess of protein is perhaps catabolised to provide energy for growth thus depressing the protein conversion efficiency. This is in concurrence with the observation of negative effects of higher dietary protein levels on the growth in plaice by Cowey et al. (1972). Maximum protein addition in the muscle of the experimented fish occurred with the diet D_2 but not with the diet D_3, suggesting that protein is maximally utilized for tissue build up at 35% protein level. The relatively poor growth at 40% protein level in the present study indicates that protein beyond an optimum level of 35% is poorly utilized for growth, as there were no significant variations observed in between the two treatments D_2 and D_3.

Table 4. Proximate composition of muscle tissue of *Labeo rohita* (% wet weight)

Parameters	Initial	D1	D2	D3
Moisture	82.46	80.24 ± 1.12^{a}	77.39 ± 1.19^{a}	78.22 ± 1.76^{a}
Protein	9.88	11.74 ± 0.05^{a}	13.32 ± 0.35^{b}	12.93 ± 0.82^{ab}
Lipid	2.8	3.32 ± 0.14^{a}	4.44 ± 0.12^{ab}	3.85 ± 0.16^{a}

The results are means ± S.D. and the figures with same superscripts in the same row are not significantly different ($P < 0.05$)

Protein digestibility was generally high and unaffected by the dietary protein level in the test Diets D_2 and D_3 and the low APD value by the diet D_1 may be due to the comparatively high carbohydrate level in the Diet, as opined by Singh and Nose (1967); Page and Andrews (1973); Shimeno et al., (1979) and Wee and Tacon (1982). In the present experiment, conducted to test the effect of animal protein incorporated formulated diets on the growth and nutritive value of rohu fingerlings, the test diet D_2 (35% dietary protein level) showed better performance in growth and feed utilization than the control diet having only plant protein and also the test diet D_3, having a higher dietary protein level. This infers that the plant protein (GOC) can be replaced by squilla meal on isonitrogenous basis and used to the maximum level of 35% protein level for better growth.

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