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Evaluation of growth response and body composition of rainbow trout, *Oncorhynchus mykiss* fingerlings fed diets containing low cost locally available feed ingredients

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Abstract
High cost of fish meal for fish feed formulation is a major constraint to the growth of aquaculture. Dearth of information on suitable alternatives to fish feed ingredients persists. A growth trial for a period of 120 days was conducted to study the effect of various locally available protein sources as replacers of fish meal in the diet of rainbow trout fingerlings. Fingerlings of mean weight 7 g were subjected to various treatments with three replicates using a Completely Randomized Design and were fed with various protein sources derived from locally available feed ingredients. On the basis of observed growth rate, Feed Conversion Ratio and Protein Efficiency Ratio, diets T1 (50% Fish Meal + 50% *Gammarus* powder) and T2 (50% Fish Meal + 50% Silkworm Pupae Meal) produced significantly best growth while the diet T8 (100% lentils + Silkworm waste) produced the lowest. The body composition also differed significantly with the highest protein and lipid deposition recorded in T1 (16.64±0.00882%) and T7 (9.27±0.00882%) respectively.

Keywords: Fingerlings, replicates, *Gammarus*, silkworm, lentils

Introduction
Aquaculture has been found to be an important approach for increasing fish production in order to make enough fish available to the world. The major constraint the use of fish meal in fish diet production has increased dramatically as aquaculture and fish feed production has grown. It has been predicted that fish meal use by aquaculture industry will decrease in the near future because prices for fish meal will increase at the same time when market prices for farmed fish decrease; forcing the fish feed industry to replace portions of fish meal in feed formulations with less expensive ingredients. Over the past few decades numerous studies focused on assessing the potential to reduce fish meal levels in the fish diets (Nandeesha *et al*., 1990; Kaushik *et al*., 1995) [15, 12]. Many plant and animal origin feed stuffs have been tested with an aim to reduce the use of fish meal in the aquaculture diet formulations. Successful results have been reported for the use of a variety of plant protein (Ogino *et al*., 1976; Higgs *et al*., 1982; Olvera *et al*., 1988) [17, 9, 18]. Similarly animal by products such as slaughter house waste, poultry offal meal have also been used as partial or full replacements of fish meal in fish feed (Borthakur *et al*., 1998) [3]. The present study was undertaken to examine the effects of certain feeds prepared from locally available ingredients on the growth performance of Rainbow trout (*Oncorhynchus mykiss*) fingerlings.

Materials and methods
Diet formulation and preparation:
Nine isoproteinous experimental diets were formulated to contain 45.12 g/kg protein and 12.25 g/kg lipid (Table 1). Chromic oxide was used as an inert marker at a concentration of 5g/kg diet. The experimental diets were prepared by mixing the dry ingredients in a food mixer and then blending with fish oil, moistened to produce a dense paste, pelleted through a hand pelletizer and dried in a hot air oven at 50-60 ^{0}C. Dried pellets were then packed in air tight plastic bags, labeled according to treatment and stored for further use.

Table 1: Formulation and proximate composition of experimental diets (g %)

Ingredients	Control	T1	T2	T3	T4	T5	T6	T7	T8
Fish meal	46.9	26.3	26.7	26.8	26.8	-	-	-	-
Gammarus powder	-	27.4	-	-	-	46.2	-	-	-
Earthworm meal	-	-	-	20.5	-	-	-	45.2	-
Kale	-	-	-	6.3	-	-	-	20.18	-
Silkworm pupae	-	-	26.8	-	-	-	46	-	-
Lentils	-	-	-	-	12.6	-	-	-	20.9
Silkworm waste	-	-	-	-	14.3	-	-	-	29.6
Soybean meal	32.5	26.1	26.3	26.2	26.2	33.6	33.8	14.5	29.3
Whole wheat	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Fish oil	8.04	8.04	8.04	8.04	8.04	8.04	8.04	8.04	8.04
Vitamins	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Yeast	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Sodium alginate	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54	0.54
Choline chloride	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Table 2: Proximate composition of formulated feeds

	Control	T1	T2	T3	T4	T5	T6	T7	T8
Dry matter	93.79	93.8	93.56	93.26	93.69	93.57	93.76	93.08	93.53
Crude protein	43.11	45.02	45.21	45.13	42.37	45.20	45.18	42.39	42.36
Lipid	12.25	12.29	13.02	12.24	12.27	13.25	12.37	12.28	12.25
Ash	15.12	14.24	13.22	15.26	15.37	14.26	15.31	14.25	15.29

Experimental procedure

The experiment was carried out in an indoor system composing of 27 FRP tanks of 1000 l capacity connected with individual water supply channels. The water flow rate in each tank was maintained at 10 l/ min throughout je experimental period. Fingerlings of Rainbow trout, *O. mykiss* used in this experiment were procured from Laribal hatchery. The animals were acclimatized for a period of 2 weeks during which they were fed with experimental diets containing 45% protein and 12% lipid.

Fingerlings with mean initial weight 7.51±0.24 g were randomly selected and distributed in 27 FRP tanks for the growth trial (30 fingerlings/ tank). Fingerlings were hand fed with the test diets at 3% body weight, thrice daily during summer months and twice in the winter months. The experimental fingerlings were weighed at fortnightly intervals for feed adjustments. The tanks were cleaned every 30 days and in order to maintain the water quality all the excess feed and faecal matter was removed. At the end of the experiment, a total of 739 fingerlings were killed and frozen immediately for growth response and body composition.

Chemical analysis

Chemical analysis of the feed ingredients, formulated diets and fish flesh was performed according to AOAC, 1995 [1]. Tissue samples were freeze dried and homogenized before analysis. Protein was determined by measuring nitrogen (N=6.25) using the Kjeldahl's method, lipid by ether extraction using Soxhlet apparatus and ash by combustion at 550^0 C for 12 hours.

Statistical analysis

All data were analysed using one way Analysis of Variance (ANOVA) using the SPSS 16.0 for Windows. Duncan's Multiple Range Test (DMRT) was performed at a significance level of $P \leq 0.05$at 95% confidence limit to know the significant difference between the treatment means for different parameters.

Results

Diets and proximate composition

Chemical analysis of the experimental diets indicated that the formulated protein levels were achieved. Desired crude protein and lipid levels in the diets were 45.12 g/kg and 12.25 g/kg respectively.

Growth, feed and protein utilization

The Rainbow trout fingerings were observed to be in good condition of health and survival was 100% in all the dietary treatments. The results of growth performance and efficiencies of protein utilization of fingerlings fed with diets containing different protein sources are presented in Table 2. Towards the end of the experiment a significant decrease in weight was observed which can be due to the decrease in the feed intake owing to decrease in temperature. The highest mean weight gain was observed in T1 (29.72±0.0033 g) followed by T2 (28.81±0.012 g) followed by Control (27.92±0.0133 g). The growth in T3 (25.37±0.0066 g), T4 (25.34±0.2082 g) was almost similar and did not vary significantly while T6 (21.24±0.0088 g), T7 (21.24±0.0088 g) and T8 (21.21±0.0133 g) showed significantly lower growth.

Specific Growth Rate (%/day) followed the same trend as that of growth showing highest value in T1 (3.37±0.0044%/day) followed by T2 (3.34±0.0188%/day), control (3.31±0.0192%/day), T3, T4 and T5 (3.21±0.0100%/day), T7 (3.04±0.0112%/day) and lowest value in T6 and T8 (3.03±0.0172%/day). Feed Efficiency Ratio (FER) also varied significantly ($P \geq 0.05$) among all the treatment groups. The mean values of FER varied from 0.46±0.0036 (T1) to 0.29±0.0064. Protein Efficiency Ratio (PER) values varied within a range of 0.49±0.004 (T1) to 0.30±0.0076 (T8) which were also significantly different in all the treatments.

Table 3: FCR, FER and PER of *Oncorhynchus mykiss* fingerlings after 120 days

Treatment	Feed Conversion Ratio (FCR)	Feed Efficiency Ratio (FER)	Protein Efficiency Ratio (PER)
Control	2.32±0.057	0.43±0.005	0.45±0.0011
T1	2.13±0.044	0.46±0.0036	0.49±0.0042
T2	2.22±0.042	0.44±0.0061	0.47±0.0064
T3	2.64±0.056	0.37±0.0028	0.40±0.0031
T4	2.65±0.046	0.37±0.0096	0.40±0.0102
T5	2.65±0.053	0.37±0.0122	0.40±0.0136
T6	3.42±0.041	0.29±0.0084	0.31±0.0088
T7	3.42±0.044	0.29±0.0064	0.31±0.0068
T8	3.44±0.053	0.29±0.0072	0.30±0.0076

Body composition of fingerlings

The final body composition of the tissues of rainbow trout fingerlings at the end of the experiment are given in Table 3. Highest and lowest moisture content was found in control (73.64±0.0033) and T6, T7 groups (72.22±0.0152), (72.22±0.0088) respectively. The values of moisture at the end of the experiment were found to be lower than the values obtained at the initiation of the experiment (78.12%). The crude protein content of the various groups varied from (16.64±0.0152%) in T1 to (16.42±0.0088%) in T7. The total lipid content of the various groups ranged from (9.27±0.0088%) in T7 to (8.34±0.0088%) in T1 and was higher than the value of lipid at the start of the experiment (5.21%). The total ash content at the end of the experiment was also higher (2.56±0.0115%) in control than at the start of the experiment (2.27%).

Table 4: Body composition of fingerlings after 120 days

Treatment	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)
Initial fish	78.12	15.04	5.21	2.27
Control	73.64±0.00333	16.58±0.00333	8.53±0.00577	2.56±0.01155
T1	73.24±0.01202	16.64±0.00882	8.34±0.00882	2.45±0.01528
T2	72.51±0.01202	16.52±0.00333	9.25±0.00882	2.46±0.01155
T3	72.51±0.01000	16.51±0.00667	9.22±0.01528	2.46±0.01155
T4	73.23±0.00882	16.63±0.00882	8.37±0.00882	2.45±0.00333
T5	73.26±0.00882	16.62±0.00667	8.44±0.01155	2.47±0.00667
T6	72.22±0.01528	16.54±0.01528	9.25±0.00882	2.48±0.00667
T7	72.22±0.00882	16.42±0.01528	9.27±0.00882	2.42±0.00667
T8	73.25±0.00667	16.61±0.01155	8.42±0.00882	2.51±0.12022

Discussion

In the present study, diets containing 50% *Gammarus* powder and 50% fish meal showed the best results in terms of growth (29.72±0.0033), feed conversion (2.13±0.044), protein efficiency (0.49±0.0042). In an experiment conducted by Vinson *et al*., 2008 [26], *Gammarus* proved to be a better source of protein when compared to mud snails and the growth of the Rainbow trout was also better, indicating that the fish is able to assimilate this amphipod crustacean in a better way. The growth performance of the fish fed with diets containing silkworm pupae clearly showed that Rainbow Trout fingerlings could assimilate non defatted pupae effectively (28.81±0.1202) and grew even better than the fingerlings fed fishmeal based control diet (27.92±0.0133). In diets for catfish (*Heteropneustes fossilis*), silkworm pupae could be used as an alternative protein source and up to 75% of the dietary protein in catfish could be replaced without affecting growth (Hossain *et al*, 1991) [10]. In the present study, experimental diets containing dried and powdered *Eisenia foetida* (earthworm) meal at levels of 50% and 100% were used to replace fish meal in the diet of Rainbow Trout fingerlings in a 120 day feeding trial. The results showed that dried earthworm meal adequately replaced the fish meal component of the control diet at low i.e. 50% (T3) and high i.e. 100% (T7) level of inclusion. There was no adverse effect on the growth performance, survival rate and feed utilization efficiency of the rainbow trout fingerlings over the entire trial period. However, at high level of inclusion i.e. when earthworm meal completely replaced the fish meal (T7), the growth performance of Rainbow Trout decreased (21.24±0.0088) and was comparatively lower than that of T3 (25.37±0.0066). Thus, the general trend which emerged from the results of the preliminary study (Tacon *et al*. 1983) [23], in which the growth performance of Rainbow Trout decreased with increasing dietary inclusion of dried earthworm meal continued.

The results of the present study indicated that the inclusion of silkworm dregs and lentils in large amounts resulted in poor growth of Rainbow Trout fingerlings (21.21±0.0133). The Feed Conversion Ratio (3.44±0.053), Feed Efficiency Ratio (0.29±0.0072) and other parameters were also lower as compared to the other experimental ingredients used. The results suggest that lentils and silkworm dregs can be used as replacements for fish meal at lower levels. The results are supported by several authors who also suggested the use of these ingredients at lower levels especially in the diets for carnivorous fish. Mondal and Kaviraj (2011) [14] studied the effect of mulberry leaf meal along with fish offal meal in the diet of freshwater catfish, *Heteropneustes fossilis.* The results of their study indicated that mulberry leaf meal can be effectively used as an ingredient to replace fish meal in the diet of catfish at a dietary inclusion of 35%.

The use of lentil seed meal as a protein source in aqua feeds has not been reported till date. However the use of pulses has been reported by several workers (Gouveia *et al*., 1993; Stickney *et al*., 1996) [8, 21]. In a ten week feeding trial period with Tilapia (*Orechromis niloticus*) fry (2.9 g), the bets daily weight gain and feed conversion was obtained at 25% level of green gram substitution. In diets for Rainbow Trout (*Oncorhynchus mykiss*), faba beans have a potential as a partial replacement for fish meal. At high levels of

replacement with faba beans (45%), supplementation with amino acids are needed (Tiews, 1981) [25].

The results in the present study showed that the amounts of different nutrients in the fish body increased as the fish grew in size. This is supported by Sutton *et al*., 2000 [22] who reported that the amounts of different chemical components within the fish body will usually increase as the body mass of the fish increases. The results of body composition also indicated that as the lipid levels of the fish fed with diets containing more lipid diet (8.34±0.0088). Fish fed on lipid rich feeds will usually be found to deposit more storage lipid than those fed on leaner diets (Manthey *et al*., 1988; Cho and Cowey, 1993) [13, 7]. The sum of the proportions of body lipid and moisture is often calculated as 80% and this seems to apply across body compartments, organs and tissues and species (Jobling *et al*., 1998) [11]. Thus, there is a strong negative correlation between the percentages of body lipid and body moisture, and this relationship seems to hold under different conditions of feeding, growth and gonad development (Weatherley and Gill, 1987; Shearer, 1994) [27, 20]. There is a strong inverse relationship between percent moisture and percent lipid in Rainbow Trout fed either high or low protein feeds at different ration levels (Reinitz, 1983) [19].

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