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Length-Weight and Feeding Indices of Formulated Diet Supplemented with Millet Base and Combined with Commercial Probiotics Used as Feed for Cultivable Freshwater Fish, *Labeo rohita*

Reka E., Kumaran T. and Saravanan R.*

Department of Zoology, Dr. Ambedkar Government Arts College, Vyasarpadi, Chennai - 600039, Tamil Nadu, India

**Corresponding Author*

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Abstract: The nutritional indices of agro based millet feed conversion was determined based on improved nutrient content with commercial probiotic raised in the semi-intensive method which was formulated by four isonitrogenic diets and given as a trail feed to Indian major carp, *Labeo rohita.* The fish were fed on groundnut oil cake, pearl millet, finger millet as base feed and a combination of pearl, finger and foxtail millet. The experimental period was 90 days. Nutritional and protein values indices values have improved and proved to be remarkable in the experimental fish and suggesting that this feed may be a better alternative feed for fish reared during aquaculture practices.

Keywords: Pearl millet, Finger millet, Foxtail millet, *Labeo rohita,* Feed indices, Protein indices, Fingerlings

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Introduction

In India fish play an important role in nutritive value for human. The better way to improve our nutritional need is to include fish in our diet. In the last five decades India has tremendous production and growth of fish. The availability of fish in India per capita is now 9.5 kg. Fish is one of the main sources of protein with all essential amino acids, fats and has become a healthier alternative to livestock during the last fifty year (Hana *et al.,* 2009). The world's most important type of aquaculture is rearing carps group in terms of production. The freshwater fish Rohu (*Labeo rohita)* is one of the most popular species especially in Asia that has high market price. Over all global production is more than 1.5 million tons in 2012 (FAO, 2013).

Dietary protein is the major and most expensive component of formulated aquafeed. Fishmeal is generally considered to be the most ideal protein source for aquatic animals, despite its static global production, seasonal/geographical variability in quality and composition. Over the past decades, there has been rapid increases in global aquaculture with competition demand and

increase in price as well, for this valuable feedstuff, with a growing interest in non - fish meal protein source for aquafeed (Pengli *et al.,* 2008).

Recently the use of millet (*Pennistum americanum)* is becoming prominent in fish feed formulation as energy source because of its abundance and relatively low price. According to Raily (2004) millet is an alternative feed grain grown extensively around the world and is superior to other cereals like maize and sorghum with respect to nutrients, especially average protein, minerals and fat (Sharma and Kapoor, 1996). Some probiotics compete with dietary dependent pathogenic bacteria and the supplementation of putative probiotics helps better utilization of feed thereby enhance the growth (Ghosh *et al.,* 2003; Wang and Xu, 2006; Wendy *et al*., 2009). Probiotics have also been found to enhance the disease resistance, and thus, could be alternatives to antibiotics and other drugs (Das *et al*., 2013; Kumaree *et al*., 2015).

Fish culture practice is improving in recent days and production is also high due to the use of supplementary feed. Research findings have shown that supplementary feed sources improve income generation. This study determined the effect of millet varieties and probiotic supplementation diet to *Labeo rohita* instead of routine fish meal in its diet and to ascertain alterations in length - weight, feeding and protein indices which was monitored throughout the period of the study.

Materials and Methods

Diet formulation and feed preparation from feed ingredients:

The present experiment involved the maintenance of fish under standard laboratory conditions throughout the experimental period. The diets were prepared by the method of Jayaram and Shetty (1981). Agro based ingredients are Pearl millet (*Pennisetum glaucum* – Kambu), Finger millet (*Eleusine coracana* – Ragi), Foxtail millet (*Setaria italic* – Thinai), rice bran, corn flour, wheat flour, soyabean flour, dried coconut (Kopari), and commercial probiotic were procured from local grocery shop at Redhills, Chennai. The cereal grains were soaked and left overnight. They were dried in sunlight, grinded finely and sieved using tea filter and nylon meshes. Mixed the right proportion of sieved ingredients with the probiotic as an additional supplement. Weighed amounts were mixed thoroughly using a common blender and mixer. The powdered ingredients were mixed with corn flour which was used as the binder in preparation of control and experimental diet (Saravanan *et al.*, 2017). A pinch of commercial probiotic was added to the feed supplement. Everfresh pro is rich in probiotics (BIOFLOC) containing naturally occurring bacteria (*Bacillus subtilis, Bascillus licheniformis, Bacillus megaterium, Bacillus pumilus, Rhodococcus* spp.).

Finely ground ingredients were mixed thoroughly with required amount of water and corn flour was added to make thick smooth dough. The dough was mixed thoroughly to ensure uniform dispersal of ingredients before pelletization. The dough pellets (2 mm diameter size) were prepared by a hand pelletizer. After sun drying, they were stored in air tight containers and kept in refrigerators until further use (Malik and Malik, 2011). The control and the experimental diet were formulated as per the composition given in Table 1.

Collection and maintenance of experimental animals:

Active and healthy fingerlings of major carp *Labeo rohita* was collected from Tamil Nadu fish seed farm, Budur, Poondi, Thiruvallur District and were brought to the laboratory in polythene bags containing aerated water. The fish were maintained in circular plastic tubs without any soil base. The tubs were washed thoroughly with water before stocking. The tubs were filled with de-chlorinated water. Fish were randomly assigned to the tubs for laboratory acclimatization for a period of 15 days. They were fed normal fish feed twice a day in the morning and evening. Left-over feed if any was removed by siphoning, two

Table 1: Composition of formulated feed (in percentage) for control and experimental fish (Ingredients refer to every 100 g of feed prepared)

S. No.	Feed Ingredients	Control	Experimental feed - I	Experimental feed - II	Experimental feed - III
1	Groundnut oil cake	100	-	-	-
2	Corn flour	-	15	15	15
3	Rice bran	-	15	15	15
4	Wheat flour	-	15	15	15
5	Soyabean	-	20	20	20
6	Coconut (Kopari)	-	04	04	04
7	Probiotic	-	01	01	01
8	Pearl millet (Kambu)	-	30	-	10
9	Finger millet (Ragi)	-	-	30	10
10	Foxtail millet (Thinai)	-	-	-	10

Values are expressed as g/100g. Probiotic values are expressed as CFU/g

hours post-feeding. The water from the fish maintenance tubs was changed thrice in a week and replenished with dechlorinated water. The accumulated feces from the bottom were siphoned out daily. Continuous oxygen supplies were provided through aerator and were maintained at 12 h light/ 12 h dark regime.

Experimental Design:

Acclimated fingerlings of *Labeo rohita* (6.60 ± 0.30 to 6.90 ± 0.10 g; 5.75 ± 0.01 to 6.57 ± 0.02 cms) were divided into four groups (consisting of 10 fishes in each group) and were given following feed:

Group I (Control feed): Fish were fed with groundnut oil cake feed for a period of 90 days (initially 2% for two week followed by 5% of the body weight twice daily).

Group II (Experimental Feed – I): Fish were fed with formulated test feed -1 for a period of 90 days (initially 2% for two week followed by 5% of the body weight twice daily).

Group III (Experimental feed – II): Fish were fed with formulated test feed - 2 for a period of 90 days (initially 2% for two week followed by 5% of the body weight twice daily).

Group IV (Experimental feed – III): Fish were fed with formulated test feed - 3 for a period of 90 days (initially 2% for two week followed by 5% of the body weight twice daily).

Fish were handled with a clean hand net. Fish were equally distributed in the tubs with two replication for each diet treatment. Aeration was continuously provided by air compressors through air stones. Feed was given for a period of 90 days, twice daily at 8.30 am and 6.30 pm. Feeding period was two hours. After the feeding time, the unconsumed food remaining in the tub was collected by siphoning out with a tube, causing least disturbance to the fish. About 75% of the water in the tubs was changed daily.

On the subsequent day before feeding, fecal matter accumulated in the tubs was siphoned out. The uneaten feed and fecal matter was collected, dried and weighed to calculate the feeding rate. All weighing were made in digital balance to an accuracy of 1 mg. The amount of food given, the amount food unconsumed were recorded daily before and after feeding. The body weight and length of the fishes were recorded once in 10 days. Sampling was done and the quantity of feed given was re-adjusted, after each sampling, based on the weight recorded.

Calculation of feeding indices and protein indices:

Feeding indices and protein indices were calculated according to the standard formula of Castell and Tiews (1980).

Statistical Analysis: Data are expressed as Mean ± SD. Software package SPSS 16.0 version was used to carry out the statistical analysis. Analysis of

Table 2: Effect of formulated millet feed supplemented with commercial probiotics on body weight differences of fingerlings of *Labeo rohita*

Experimental groups (Feeding Regime)	Body weight (g)		Paired sample 't' test			
	Initial (Day-1)	Final (Day-90)	Paired sample test difference	t- value	df	2-tail significance
Control feed	6.90 ± 0.10	24.90 ± 0.10	18.00 ± 0.005	5401.00	2	0.000*
Experimental feed-I	6.70 ± 0.10	26.63 ± 0.25	19.53 ± 0.15	226.02	2	0.000*
Experimental feed - II	6.60 ± 0.30	25.47 ± 0.25	18.72 ± 0.05	566.00	2	0.000*
Experimental feed - III	6.67 ± 0.15	32.36 ± 0.02	25.36 ± 0.13	334.18	2	0.000*

Values are Mean ± SD (n= 10), Statistical significance tested at 5% level:* p<0.05 (Compared between initial and final weight for each group)

Table 3: Effect of formulated millet feed supplemented with commercial probiotics on length gain of fingerlings of *Labeo rohita*

Experimental groups (Feeding Regime)	Length (cm)		Paired sample 't' test			
	Initial (Day-1)	Final (Day-90)	Paired sample test difference	t- value	df	2-tail significance
Control feed	6.57 ± 0.02	12.86 ± 0.03	6.29 ± 0.01	1089.460	2	0.000*
Experimental feed - I	6.13 ± 0.02	13.14 ± 0.03	7.01 ± 0.01	1052.000	2	0.000*
Experimental feed - II	5.75 ± 0.01	12.81 ± 0.02	7.06 ± 0.01	1222.828	2	0.000*
Experimental feed –III	5.85 ± 0.02	16.58 ± 0.02	10.73 ± 0.01	334.184	2	0.000*

Values are Mean ± SD (n= 10), Statistical significance tested at 5% level:* p<0.05 (Compared between initial and final weight for each group)

variance (ANOVA) was used to compare feed indices and protein indices between control and experimental groups and statistical significance was tested at 1% and 5% levels.

Results

Body weight and length gain:

The present study demonstrated that diet supplemented with millet combined with probiotic feed enhanced growth and length in the experimental groups when compared to control. Among the tested diet, experimental feed - III showed higher rate of growth in terms of length and weight of fish (Tables 2, 3).

Feeding indices:

Feed conversion efficiency, feeding rate, food absorbed, absorption rate and absorption efficiency was significantly increased (p<0.01) in the experimental groups when compared to control diet fed fish. A significant decline (p<0.05) was seen in feed conversion ratio of experimental

Table 4: Effect of formulated millet feed supplemented with commercial probiotics on feeding indices of fingerlings of *Labeo rohita*

Feeding indices	Control feed	Experimental feed - I	Experimental feed - II	Experimental feed - III	F- value	P – value	P-value
Feeding conversion ratio (g)	$0.07^{b} \pm 0.01$	$0.05^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.07^{b} \pm 0.01$	5.751	0.000^{**}	0.003^{**}
Feed conversion efficiency (%)	$1.45^{a} \pm 0.85$	$1.49^{c} \pm 1.96$	$1.47^{b} \pm 17.13$	$1.58^{d} \pm 29.08$	109.180	0.000^{**}	0.000^{**}
Feeding rate (g/day)	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.01$	$0.06^{a} \pm 0.00$	$0.08^{b} \pm 0.00$	22.553	0.000^{*}	0.000^{**}
Food absorbed (mg/day)	$0.57^{b} \pm 0.12$	$0.77^{c} \pm 0.12$	$0.27^{a} \pm 0.12$	$0.79^{c} \pm 0.10$	46.407	0.000^{*}	0.000^{**}
Absorption rate (mg/gm/day)	$0.03^{a} \pm 0.01$	$0.05^{b} \pm 0.01$	$0.03^{a} \pm 0.01$	$0.06^{c} \pm 0.01$	18.656	0.000^{*}	0.000^{**}
Absorption efficiency (%)	$66.60^{b} \pm 0.11$	$85.70^{d} \pm 1.16$	$42.50^{a} \pm 1.08$	$71.70^{c} \pm 1.16$	3350.00	0.000^{*}	0.000^{**}

Values are Mean ± SD (n=10); ** denotes significance at 1% level; Different alphabets between groups denotes significance at 5% level using Tukey multiple range test ($p < 0.05$), Means carrying common superscript between groups do not differ significantly

Table 5: Effect of formulated millet feed supplemented with commercial probiotic indices in fingerlings of *Labeo rohita*

Protein indices	Control feed	Experimental feed			F- value	P-value
		I	II	III		
Protein conversion ratio (PCR) (g)	$0.53^{a} \pm 0.02$	$0.72^{c} \pm 0.02$	$0.64^{b} \pm 0.02$	$0.83^{d} \pm 0.02$	537.591	0.000*
Protein conversion efficiency (PCE) (%)	$92.23^{a} \pm 0.31$	$1.11^{c} \pm 0.30$	$1.02^{b} \pm 0.28$	$1.18^{d} \pm 0.30$	13270.00	0.000*
Protein utilization (PU) (g)	$28.94^{b} \pm 0.29$	$27.91^{a} \pm 0.33$	$30.05^{c} \pm 0.30$	$31.95^{d} \pm 0.30$	319.931	0.000*

Values are Mean ± SD (n=5), *denotes significance at 1% level

diet fed fish when compared to control diet fish (Table 4).

Protein indices:

Protein conversion ratio, protein conversion efficiency and its utilization noted significant changes ($p<0.01$) in formulated diet fed group when compared to control diet fed fishes (Table 5).

Discussion

Aqua farmers are now compelled to search for cheaper alternative protein sources of plant origin. Agro products combined with commercial probiotic act as an excellent diet for freshwater fishes. Millet based probiotic combined experimental diet is used directly as value added feed supplement. Nutritional value of millets is a potential substitute of protein and other ingredients in fish feed. Millets are an excellent nutrient source, containing high amounts of macro- and micronutrients as well as bioactive compounds and high antioxidant health beneficial properties. Millet supplementation in aqua feeds may be a valuable tool to increase the health and immune competency of valuable aquaculture fish species (Wilson *et al.,* 2015).

In the present study, the suitability of formulated agro based millet combined with probiotic value added diet fish meal with complete plant protein ingredients was assessed. This effect was evaluated against normal groundnut oil cake used as control feed along with experimental feed

on cultivable fish *Labeo rohita*. The formulated feed are rich in nutrients contents (Martin *et al.,* 2016). Newly formulated millet based probiotic combined diet should satisfy the nutritional requirement of the fishes with high acceptability of the feed (Lekang *et al.,* 2017).

The present study focused to evaluate the potential of millet based experimental feed. Results on the effect of agro based feed supplemented diet increased weight and length with marked improvement in feeding and protein indices. The weight and length of the fish is directly dependent on food composition, quality and quantity and various types of feed ingredients have been tried as substitute for natural food for freshwater fishes. This may be due to the unstressful, more palatable and digestible in nature. Fish is a rich source of animal protein and to achieve maximum yield it is necessary to provide artificial feed by which fish attain maximum weight in shortest possible time (Bhosale *et al.,* 2010). Several agro based product and some herbs were analysed for the growth promoting activities (Citarasu *et al.,* 2002). The length and weight of the fish showed progressive increase from the beginning to 90 days of the experimental study period. Length-weight relationship of fish fluctuates relying on the state of life in aquatic environment and it is an imperative tool that gives data on development and its pattern of animals (Ighwela *et al.,* 2011). The pattern of growth performance was remarkable in experimental feed - III, suggesting that the diet was acceptable. The difference in weight gain in experimental fishes of different groups may be attributed to variation in the feed used and their ingredients (Ashraf *et al.,* 2008).

The control fishes were fed with groundnut oil cakes which form the basal diet. In the present study fingerlings of *Labeo rohita* fed on various millet based probiotic combined experimental diets for 90 days resulted significant increase in body weight and length when compared to fishes fed on control diet. The pattern of growth performance was remarkable in experimental diet - III, suggesting that the diet was acceptable. The difference in weight gain in experimental fish of different groups may be attributed to variation in the feed used and their ingredients (Saravanan *et al*., 2017).

The feed conversion ratio (FCR) were lower for millets combined probiotic formulated feed group when compared to that of fishes fed on control diet. The present findings are in accordance with studies carried out in *Labeo rohita* fed with millet combined with probiotic formulated diet with high protein level (Kodithuwakku *et al.,* 2017). It is proved that lower the FCR, higher is the quality of the feed as observed in many freshwater fishes where the feed conversion ratio is known to decrease with increasing dietary protein content (Ebtehal, 2017). Feeding rates affect nutrient requirements in fish. The food utilization efficiency is mainly dependent on the quality of the diet (Khan *et al.,* 2012). The present study indicated higher food absorption, absorption rate and efficiency in juvenile fishes fed on experimental diet when compared with the fish fed on a normal basal diet (Gnanavel and Saravanan, 2019).

Results obtained in this study can be regarded as an index of food utilization. It can be suggested that plant source ingredients in the diets are easily and efficiently digested and absorbed. The increase in absorption activities and its conversion efficiency could be due to an increase in digestive enzymes activity and utilization in fingerlings of *Labeo rohita* fed on agro based millet combined with probiotic formulated feed. It is suggested that the use of various dietary protein sources in combination was more effective than a single source in replacing fish meal in diet.

Protein is the most expensive dietary macronutrients and directly affects fish weight gain (Abdelghany, 2000). Fish is a rich source of animal protein and to achieve maximum yield it is necessary to provide nutritious supplemental or formulated feed by which fish attain maximum weight in shortest possible time. Higher protein

content present in the formulated feed indicated its growth promoting potential and feed conversion efficiency (FCE). It is suggested that use of various dietary protein sources in combination was more effective than a single source in replacement of fish meal with millet based probiotic diets for *Labeo rohita* which improved PER and lower FCR (Kim *et al.,* 2013). A protein level of 30% in the feeds can lead to optimal growth. Since protein has not become the source of energy provision, the protein is diverted to carry out its functions of tissue building (Aliyu *et al*., 2019)

The protein conversion ratio (PCR), protein conversion efficiency (PCE) and protein utilization (PU) was also higher in fish fed with experimental feed -III, when compared with other experimental groups. Results obtained in this study can be regarded as an index of food utilization. It can be suggested that plant source ingredients in the diets are easily and efficiently digested and absorbed. The increase in absorption activities and its conversion efficiency could be due to an increase in the activity of digestive enzymes and utilization in fingerlings of *Labeo rohita* fed on millet combined with probiotic formulated feed.

Agro based millet combined with probiotic supplementation in aqua feeds may be a valuable tool to increase the health and immune competency of valuable aquaculture fish species (Peixoto *et al.,* 2016). Dietary supplementation of multi-strain probiotics improved both survival and growth of Rohu at hatchling stages i.e. during the first month of rearing. Probiotics affect the intestinal ecosystem by stimulating mucosal immune mechanisms through competition with pathogenic microorganisms. Non-digestible nutrients including dietary fibers in the feed are further fermented by the available probiotics in large intestine and thus producing oligo-compounds as well as simpler molecules including fatty acids, which improve their uptake and enhance the growth (Jha *et al*., 2015).

Conclusion

It may be suggested from the present study that incorporation of agro based millet combined with probiotic in the feed makes the food more palatable, stimulates better consumption, improves assimilation, enhances growth and increases the protein content of the fish millet formulated feed combined with commercial probiotics. Hence, this plant based ingredients can be considered as formulated feed for freshwater fishes which are cultivable. It could be inferred that the inclusion of this agro based formulated feed as an additive in fish feed could possibly aid productivity in aquaculture.

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