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Study on Complete Replacement of Fish Meal by Plant Sources Meal as a Dietary Protein for *Labeo rohita* and *Ctenophyryngodon idella*

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Abstract.- The main objective of this study was to evaluate alternate vegetable proteins as a replacement for fish meal in diets of *Labeo rohita* and *Ctenopharyngodon idella* and use the findings to formulate a cost effective diet for freshwater fish species. Fish of an average weight of (*L. rohita* 1.91±0.05; *C. idella* 19.57±0.09 g) was stocked in four earthen fish ponds. The feed was prepared from five different plant sources (35 % crude protein). The diet was fed at the rate of 0.15 g nitrogen per 100 g of wet fish weight daily for one year. Fish were fed two times per day by dusting method and control pond remained without any additives. *L. rohita*, and *C. idella* performed in a curvilinear manner at different water temperatures. The growth performance in *C. idella* was comparatively better with the feed supplementation compared to *L. rohita*. In *L. rohita* and *C. idella* weight gain at the completion of the study, expressed as a percentage of the weight over control, pond computed as 375.69 and 356.63 percent, respectively with the effect of feed. It has been concluded that fish meal can be successfully completely replaced in the diets for these fish species without any adverse effects on fish growth performance.

Key Words: Plant protein, condition factor, *Labeo rohita*, *Ctenopharyngodon idella*, length-weight relationship.

INTRODUCTION

The demand for compound fish feed was estimated to be 29.3 million tonnes in 2008 and is expected to grow along with increased global aquaculture production (FAO, 2011). The compound fish feed production has grown at an average rate of 10.9 percent/ year since 1995 (FAO, 2011). In order to reduce the pressure on fish meal in the gradually increasing in aquaculture industry, alternative feed ingredients from the plant, microbial and other animal sources have been a prioritized field of research from last one decade. Fish feeding costs amount to 70% of the operational cost of fish farming. The shortage in global production of fish meal, which is the major traditional animal protein source combine with its increased demand in feeds for livestock, poultry and other husbandry animals, is likely to decrease the dependence on it as a single protein source in aqua feeds (El-Sayed, 1999). Fish meal is considered the most advantageous animal protein ingredient in aquaculture feeds because of its high protein content, balanced amino acid profile, high digestibility and palatability, and as a source of essential n-3 polyenoic fatty acids (Hardy and Tacon, 2002). Therefore, fisheries scientists have made a multiple efforts to partially or totally replace fish meal with less expensive and locally available protein sources, such as legumes (De Silva *et al*., 1988), lupine seed meal (De La Higuera *et al*., 1988), soybean meal (SBM) (El-Saidy and Gaber, 1997; Eric *et al*., 2000; Carter and Hauler, 2000), cottonseed meal (El-Sayed, 1999; El-Saidy and Gaber, 2004), cluster bean meal (El-Saidy *et al*., 2005), faba bean meal (Deyab *et al*., 2006) and cow pea seed meal (Deyab and Aml Saad, 2008) sesame seed meal (Deyab *et al*., 2009) which were reported to be suitable as a partial replacement for fish meal. Many different plants-protein sources have been examined, including plant-protein meals and plant-protein concentrates (Lim *et al.*, 2008). Inclusion of feedstuffs with relatively high levels of carbohydrate in formulating fish feed is preferred in view of its protein-sparing action that may make the diet most cost effective (Hidalgo *et al.*, 1993). Bhosale *et al*. (2010) reported that an increased use of plant protein supplements in fish feed can reduce the cost of fish meal. The research has focused on utilizing less expensive and readily available

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resources to replace fish meal, without reducing the nutritional quality of feed (Mahboob and Sheri, 1997). There is less information available on the use of plant as an alternative source for preparation of fish feeds. The purpose of this study was to test plant protein ingredients as a replacement for fishmeal in diets for *L. rohita,* and *C. idella* and to use these results to develop a cost effective ration for fish.

MATERIALS AND METHODS

Four newly dug earthen fish ponds of dimensions 15m x 8m x 2.5m (length x width x depth) were used for this investigation. All the ponds were sun-dried for a period of fifteen days. In order to ensure disinfection of these ponds, liming was done with CaO at the rate of 2.40 kg/pond (Javed, 1988) with a dusting method. The inlets of all these ponds were properly fixed with screen to avoid the entry of any intruder into or exit of fish from the ponds. All the ponds were filled with un-chlorinated tube well water up to the level of 2.0 m and this level was maintained throughout the experimental period so as to have a pond volume of 240.00 m^3 for fish cultivation. A total of 84 four-months-old fingerlings of *L. rohita* (weight 1.91±0.05 g, fork length 50.84±0.08 mm*)* and *C. idella* (weight 19.57±0.09g, fork length 93.16±0.07 mm) were stocked in each of the ponds with a stocking density of 2.87 m^3/fish (Javed, 1988). The interspecies stocking ratios for *L, rohita* and *C. idella* was 60:40.

Preparation of feed

The plant protein feed (35% crude protein) was prepared from five different ingredients, sesame oil cake (SOC; 32%), maize gluten meal (MGM; 20%), cottonseed meal (decardicated; CDM; 40%), wheat bran (WB; 3.5%), rice polish (RP; 3.5%). SOC, MGM, CDM, WB and RP were combined with vitamin and mineral mix to produce a fish feed that contained digestible protein levels similar to menhaden fishmeal (select grade). Fish feed was prepared using commercial manufacturing technology as described earlier by Mahboob (2014). The feed was stored in plastic lined paper bags at room temperature until fed. The field trial was conducted with three replicates and one control pond. The feed was fed at the rate of 0.15 g nitrogen per 100 g of wet fish weight daily for one year. Each pond was stocked with a total 84 fish with three replicates, arranged in a completely randomized design. Fish were fed twice a day, whereas, control, pond remained without any additives.

Growth studies

The cultured fish stock was sampled, randomly, with three repeats on every 16th day (designated as fortnight) by using nylon drag nets from each of the ponds during the 12-months trial. The morphometric characteristics of fish *viz.*, wet body weight, wet fork length and wet total length were measured and recorded to monitor growth and released back into their respective ponds. The sample size for each fish species remained 7 as determined by the following formula:

$$N = t^2s^2/d^2$$

t is Value of t from the normal probability table against α = 0.05; s^2 is the variance among units in the population from previous work (Javed, 1988), and d is desired margin of error in the estimate (one percent)

Statistical analysis

The data were subjected to statistical analysis by using Minitab software. The differences among treatments were tested using ANOVA followed by the DMR test.

RESULTS AND DISCUSSION

The final average weight after trial 12 moths in *L. rohita* and *C. idella* in treated and control ponds were recorded as 601.42±2.54, 160.08±0.29 and 1167.85±3.79 and 327.46±3.29 g, respectively (Table I, Figs. 1-2). In *L. rohita* a gradual increase in weight was observed from the beginning of the experiment to the end of the 24th fortnight (December). Figure 1A shows a significant increase in the body weight of *L. rohita* fed on supplemented diet as compared to control, particularly from first to sixteen fortnights. The weight increase after 17th fortnight was gradually slower down till the end of

Table I.- Body weight (g) and fork length (mm) of *L. rohita* and *C. idella* in control and feed supplemented pond recorded every fortnight for a period of 12 months.

L. rohita				*C. idella*			
Feed supplemented pond		Control pond		Feed supplemented pond		Control pond	
Body weight	Fork length	Body weight	Fork length	Body weight	Fork length	Body weight	Fork length
3.23±0.02	73.24±0.5	2.06±0.02	54.31±0.3	25.[illegible]0.9	105.12±0.4	21.49±0.5	98.93±0.4
7.18±0.04	87.17±0.6	2.98±0.03	65.52±0.2	34.12±1.1	127.73±0.5	24.34±0.7	112.87±0.5
12.65±0.03	99.57±0.7	3.97±0.01	71.69±0.4	58.41±1.3	140.67±0.7	29.46±0.5	125.72±0.7
21.42±0.05	106.68±0.8	5.04±0.04	76.82±0.5	93.23±1.5	150.04±0.9	35.07±0.7	131.02±0.9
34.14±0.04	140.83±0.9	7.51±0.05	90.28±0.6	133.81±1.6	186.89±1.2	43.84±0.9	141.65±1.1
51.82±0.5	146.19±1.1	11.78±0.2	99.38±0.8	178.99±1.7	200.29±1.5	53.21±1.0	147.46±1.3
70.26±0.4	162.39±1.4	16.89±0.3	105.63±1.0	230.37±1.5	224.38±1.6	64.15±1.1	153.34±1.5
93.34±0.6	171.78±1.2	22.28±0.4	109.12±1.2	285.13±1.7	253.05±1.8	76.69±1.3	159.19±1.8
118.06±0.8	173.94±1.4	29.62±0.5	114.89±1.3	344.72±1.9	275.48±1.9	93.56±1.5	166.89±1.6
147.43±0.6	176.03±1.3	37.44±0.6	130.71±1.4	415.55±2.2	296.77±2.2	116.93±1.6	179.26±1.8
179.78±0.7	180.77±1.5	46.13±0.5	147.08±1.2	489.68±2.5	331.14±2.3	147.29±1.7	192.96±1.9
212.94±0.9	199.17±1.7	55.99±0.9	153.11±1.6	594.36±2.7	334.82±2.5	174.62±1.9	200.04±2.2
247.55±0.8	208.25±1.6	66.38±0.8	164.22±1.8	689.84±2.9	354.22±2.6	199.37±2.1	206.59±2.3
285.43±0.7	220.46±1.7	79.25±1.0	170.57±1.9	780.79±3.3	367.34±2.4	220.19±2.2	212.41±2.5
343.11±0.9	245.79±1.9	96.58±1.1	172.66±1.6	853.27±3.6	378.53±2.8	239.03±2.4	215.66±2.8
384.37±1.1	251.05±2.1	109.85±1.3	179.49±2.0	908.46±3.8	386.16±3.0	256.86±2.6	219.29±2.4
421.03±1.2	255.55±2.2	121.28±1.5	182.15±2.2	957.93±3.5	391.42±3.2	272.32±2.9	222.74±2.8
455.49±1.3	264.22±2.1	131.38±1.7	189.74±2.3	1001.71±3.9	404.91±3.4	285.47±2.7	225.37±2.6
486.97±1.2	282.62±2.3	140.13±1.5	193.32±2.5	1042.05±4.3	407.75±3.6	296.72±2.9	228.05±2.7
517.83±1.3	286.96±2.5	148.45±1.9	194.51±2.6	1079.64±4.5	410.64±3.9	305.99±3.2	231.81±2.9
543.15±1.5	289.13±2.9	154.21±2.2	195.92±2.8	1112.25±4.6	413.08±4.2	313.09±3.4	233.62±3.0
566.38±1.6	294.33±3.1	157.83±2.4	197.85±2.5	1133.46±4.4	415.71±4.3	319.28±3.3	235.12±2.8
585.52±1.8	296.88±3.3	159.79±2.5	198.24±2.7	1151.67±4.8	417.32±4.5	324.17±3.4	238.51±3.4
601.42±1.9	299.59±3.5	160.08±2.7	199.50±2.9	1167.85±4.5	419.85±4.7	327.46±3.2	240.03±3.7

study both in control and supplemented ponds. *L. rohita* obtained a maximum increase (57.68 g) during the 15th fortnight (Fig. 1A). *C. idella* obtained a maximum increase 104.68 g and 30.36 g in its average weight during the 12th and 11th fortnight, respectively at feed and supplemented and control ponds (Table I, Fig. 1B). *L. rohita*, and *C. idella* performed in a curvilinear manner at different water temperatures. *C. idella* comparatively responded better with the feed supplementation compared to *L. rohita*. In *L. rohita* and *C. idella* weight gain at the completion of the study, expressed as a percentage of the weight over the control point, was computed as 375.69 and 356.63 percent, respectively, with the effect of feed prepared from plant sources by replacing the fish meal.

The experiment was started with the initial average fork lengths of 50.84±0.08 and 93.16±0.07 mm of *L. rohita* and *C. idella,* respectively (Table I). At the end of experiment, *L. rohita* and *C. idella* attained an average total length of 299.59± 3.5 and 419.85±4.7 mm; 199.50±2.9 and 240.03±3.7 mm in treated and control ponds, respectively (Table I). The maximum increase in fork length in *L. rohita* was recorded during the 5th fortnight as 34.15 mm and 16.37mm during 11th fortnights in feed supplemented and control ponds, respectively (Fig. 2A). The maximum increase in fork length of *C. idella* in the pond supplemented with artificial feed was recorded as 36.85 mm during 5th fortnight followed by 28.67 mm during 8th fortnight, respectively (Fig. 2B). The maximum increase in fork length of *C. idella* in the control pond was recorded as 13.94 during 2nd fortnight followed by 13.70 mm during 11th fortnight, respectively (Fig. 2B). The slow growth rate, in the beginning of the experiment in this study was due to low water temperature during colder months. Javed (1988) reported maximum growth of major carps within

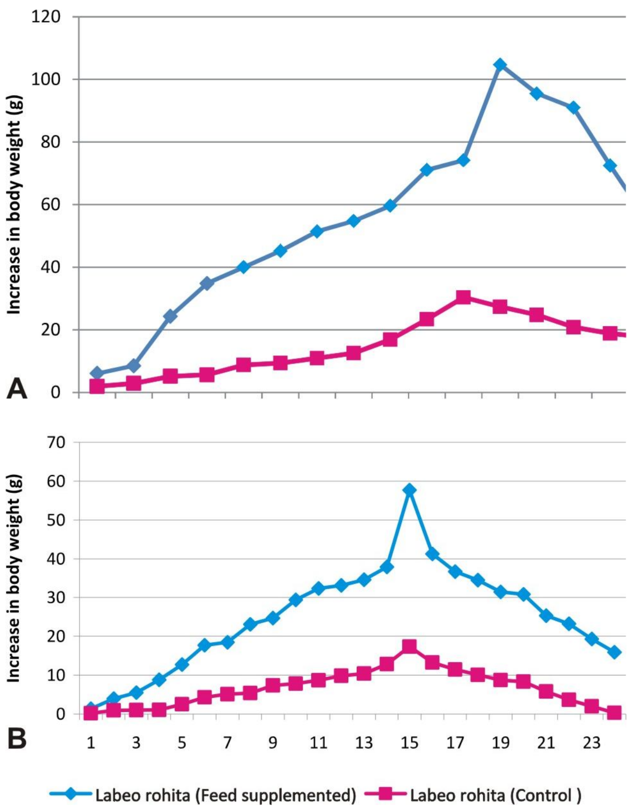

Fig. 1. Fortnightly increase in wet weight (g) in *C. idella* (A) and *L. rohita* (B) in feed supplemented and control pond.

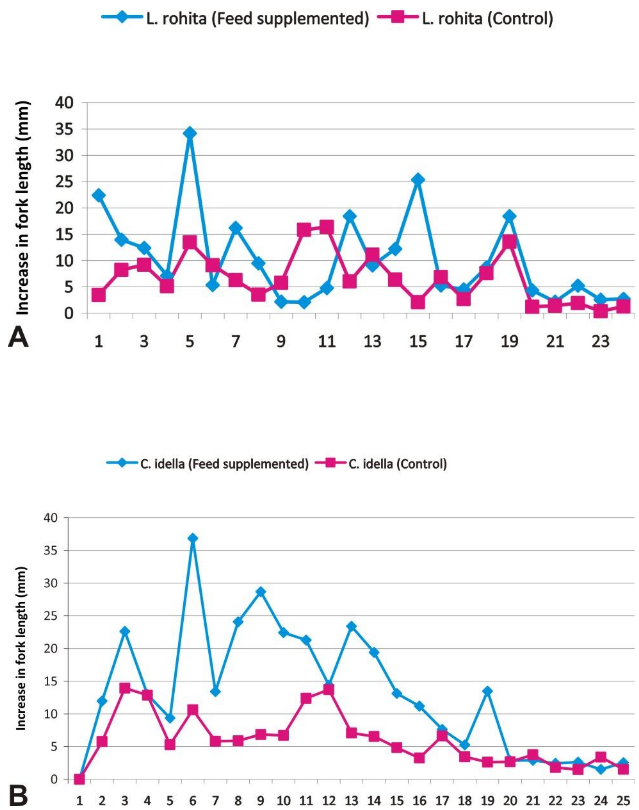

Fig. 2. Fortnightly increase in fork length (mm) in *L. rohita* (A) and *C. idella* (B) in feed supplemented and control pond.

temperature range of 30 to 33.73°C. In the present study, best growth of these fish species was obtained within a temperature range of 24.85-31.16°C. The findings of the present study are not in agreement with the findings of Barthelmes and Jahnichen (1978), who reported the growth of *H. molitrix* at water temperatures ranging from 18 to 22°C. It is quite clear from the above results that growth of fish is dependent upon food consumption and extra energy after meeting the energy needs of basal metabolism and activity of the fish. Feeding activity and food requirements and consumption patterns are affected by water temperature. In the present study warmer months found to be the best period for the growth performance of the fish. Varghese *et al*. (1980) reported that silver carp (*H. molitrix*), grass carp (*C. idella*) male common carp (*Cyprinus carpio*), rohu (*L. rohita*) and mrigal (*Cirrhina mrigala*), responded to the feed consisting of rice bran: oil cake 1:1 at 2% body weight, were in accordance with the results of the present study. It was interesting to note that *L. rohita* is column feeder and *C. idella* feeds on vegetation, but latter performed better on supplementary feed in a polyculture system which contradicted the results of Chakarabarty *et al*. (1976). The left over feed might have improved the growth of vegetation and phytoplankton life.

Length-weight relationship and condition factor

Length-weight relationship is of great importance in fisheries. This relationship of fish has often been studied biologically. Length is considered as an independent variable while weight as a dependent one. The main objective of the length - weight relationship is to determine the variation from the expected weight of fish or a group of a fish to express the condition of fish in numerical terms (degree of well-being, relative robustness, plumpness or fatness). This relationship is called the coefficient of condition or condition factor. It was calculated from length-weight data for comparison of the condition of fish under the influence of various treatments. According to LeCren (1951) the length-weight relationship would first be calculated as the logarithmic formula:

$$\log W = \log c + n \log L$$

The length-weight equations were fitted to the mean values obtained from the weights (g) and fork length of sample fishes of each species at each fortnight. The values of c and n were obtained through the computer. From these values calculated weights were determined for a known mean fork lengths for which the mean observed weight were also known. In the present study the relative condition coefficient (Kn) which is independent of the units of measurements has been obtained by the formula:

$$Kn = W / w$$

Where "W" is observed weight and "w" is the calculated weight of fish.

Table II.- Fork length –weight relationship for *L. rohita* and *C. idella* in feed supplemented and control ponds.

Species	Treatment	Regression equation	r
L. rohita	Feed supplementation	log W= -4.42+2.590 log F.L (0.300)	0.949
	Control	log W= -5.80+3.471 log F.L (0.065)	0.996
C. idella	Feed supplementation	log W= -4.05+2.709 log F. L (0.048)	0.999
	Control	log W= -5.86+3.525log F. L (0.085)	0.994

Values within brackets are standard errors; W: fish weight; F.L: fork length

The regression equations for fork length-weight relationships of fish species is presented in Table II. The high values of "r" for regression equations at each treatment level indicated reasonable precision of these equations for each fish species. Length-weight relationship indicates that both the fish species did not deviate from the general trend of such relationship feed supplementation. The mean condition (K) calculated in this study revealed that the population of fish was close to 1 showed that fish was in good condition in treated ponds. The results of this study revealed that the population of *L. rohita* and *C. idella* in all the ponds followed an isometric growth pattern. These findings are substantiated by Ali *et al*. (2014). However, the differences were remained statistically significant at fortnight levels, indicating that fish deviated from the general trend of its length-weight relationship with age and season.

After one year experimental period all the ponds were harvested for final fish yield. The total production of *L. rohita* were calculated as 288.41 and 76.83 kg, in treated and control ponds, respectively. For *C. idella* the total production of 373.12 and 104.78 kg were recorded in treated and control ponds, respectively. Fish meal is one of the most expensive ingredients in prepared fish diets. Fish nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. The effects of plant protein feed and concentrates on the growth of fish have been reported by various workers (Mahboob and Sheri, 1997; Gatlin *et al.*, 2007; Lee *et al.*, 2006; Lim *et al.*, 2008; Ayse, 2011). To find an alternative protein sources to replace fish meal in fish feed is important if the growth of the fishing industry is to be sustained (Francis *et al.*, 2001; Mahboob and Sheri, 2002). There has been an increasing concern to prepare a cost-effective and practical approach to utilize alternative protein sources more efficiently in freshwater fish species towards sustainable fish farming. It has been observed in this study that both the fish species performed significantly better in terms of increase in the weight and length compared to control ponds. The better conversion efficiency of feed into fish flesh in *L. rohita* and *C. idella* is due to their feeding habit.

CONCLUSION

It has been concluded that vegetable material is a suitable source as a fish meal replacement in *L.*

rohita and *C. idella.* Grass carp performed better as compared to *L. rohita* in terms of growth performance. So, fish meal can be replaced easily with an alternative plant protein sources like cottonseed meal, maize gluten meal and sesame oil cake. Therefore, the findings of this study will encourage feed manufacturers to utilize plant proteins more efficiently in generating low-cost and sustainable fish feed.

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