Pakistan J. Zool., vol. 46(3), pp. 747-752, 2014.

Replacing Fish Meal With a Blend of Alternative Plant Proteins and its Effect on the Growth Performance of *Catla catla* and *Hypophthalmichthys molitrix*

Shahid Mahboob*
Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh-11451, Saudi Arabia.

Abstract.- Intensive fish culture practices generated a demand for an efficient, balanced and economical diet. The purpose of this study was to evaluate an alternate plant proteins as a replacement for fish meal in diets for *Catla catla, and Hypophthalmichthys molitrix* and to use the results to develop and test cost effective alternate plant protein blends in diets for freshwater fish species. The plant protein feed (35 % crude protein) was formulated from five different ingredients. The feed 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. However, the control pond remained without any additives. *Catla catla*, and *H. molitrix* performed in a curvilinear manner at different water temperatures. *H. molitrix* comparatively performed better with the feed supplementation compared to *Catla catla*. In *Catla catla* and *H. molitrix* weight gain at the completion of the study, expressed as a percentage of the weight over the control pond computed as 436.53 and 330.37 percent, respectively, with the effect of feed prepared from plant sources by replacing the fish meal.

Key Words: Fish feed, plant protein, condition factor, growth performance of fish, aquaculture production.

INTRODUCTION:

Fish is a highly nutritive and rich source of an animal proteins. For the improvement of fisheries and to achieve maximum yields from resources of fresh water, it is necessary to provide artificial feed, by which fish grows rapidly and attains maximum weight in the shortest possible time. The demand for seafood continues to increase, and aquaculture production has filled the shortfall associated with static wild fish landings (FAO, 2010). In fact, in 2012 aquaculture production is expected to exceed capture fisheries as a source of finfish products for consumption (FAO, 2010). Aquaculture production is expected to increase further and this will require higher production of aqua feeds. The inclusion of plant-protein sources in aqua feeds has increased due to the limited amount and increasing cost of fishmeal available for production of animal feeds (*e.g.,* Gatlin *et al.,* 2007; Glencross *et al.,* 2004; Naylor *et al.,* 2009). One of the greatest challenges for the aquafeed industry is to reduce fishmeal levels in feed further and increase the amount of plant protein and ingredient diversity in the feed of carnivorous fishes. Among commonly used feed ingredients, fish meal is considered to be the best ingredients, due to its compatibility with the protein requirement of fish (Alam *et al.,* 1996). Replacement of fish meal with cheaper ingredients of plant origin in fish feed is necessary because of rising costs and uncertain availability of fish meal (Higgs *et al.,* 1995). Many different plants-protein sources have been examined, including plant-protein meals and plant-protein concentrates (Lim *et al.,* 2008). Inclusion of feed stuffs 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). According to Bhosale *et al.* (2010) 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 resources to replace fish meal, without reducing the nutritional quality of feed (Mahboob and Sheri, 1997). The purpose of this study was to test plant protein ingredients as replacement for fishmeal in diets for *Catla catla,* and *Hypophthalmichthys molitrix* and to use these results to develop and test protein blends in diets to enhance production of these fish species.

* Corresponding author: shahidmahboob60@hotmail.com
0030-9923/2014/0003-0747 $ 8.00/0

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 (200 kg/ha as suggested by Hora and Pillay, 1962) with dusting method. The inlets of all the 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 unchlorinated 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 *Catla catla* (weight 2.87±0.08 g; total length 59.3±0.09 mm*)* and *Hypophthalmichthys molitrix* (weight 22.14±0.07g; total length 143.74±0.16 mm was stocked in each of the ponds with a stocking density of 2.87 m^3/fish (Javed, 1988). The interspecies ratios were, 50:50.

Preparation of feed

The plant protein feed (35 % crude protein) was formulated 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), which were combined and then 1% vitamin and mineral mix was added to produce fish feed that contained digestible protein levels similar to menhaden fishmeal (select grade). The rationale behind the protein-blend approach was that in commercial feed formulations, reducing fishmeal levels is best accomplished by combining several alternate protein sources to approximate the amino acid profile of fishmeal. Supplementing protein blends with amino acids further improved the nutritional profiles of blends in comparison with that of fishmeal (Cheng *et al*., 2003). Minerals shown to be important when feeding fishmeal-free diets were supplemented to each of the three blends (Barrows *et al*., 2010). Fish feed was prepared using commercial manufacturing technology. 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 contained 84 fish and feed fed to three replicate ponds, arranged in a completely randomized design. Fish were fed twice a day by dusting. However, the 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:

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

where t, value of t from the normal probability table against α, 0.05; s^2, the variance among units in the population from previous work (Javed, 1988); d, 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

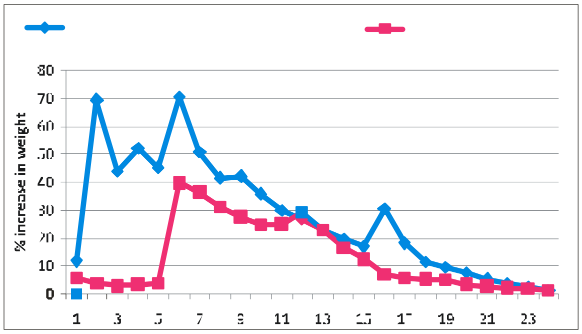
The final average weight after a trial of 12 moths in *Catla catla* and *H. molitrix* in treated and control ponds were recorded as 870.14±2.03, 162.19±1.72 and 1136.83±3.09 g and 264.15±2.14 g, respectively (Table I). Figure 1A shows a significant increase in the body weight of *Catla catla* fed on supplemented diet as compared to control particularly during the first seven fortnights. The weight increase after 7^{th} fortnight gradually decreased and was not significantly different from that of the control fish. *H. molitrix* obtained a maximum increase in its average weight during the 9^{th} fortnight in the control experiment (Fig. 1B).

Table I.- Body weight (g) and fork length of *Catla catla* and *H. molitrix* in control and feed supplemented pond recorded every fornight for a period of 12 months.

Catla catla				*H. molitrix*			
Control pond		Feed supplemented pond		Control pond		Feed supplemented pond	
Body weight	Fork length (mm)	Body weight	Fork length (mm)	Body weight	Fork length (mm)	Body weight	Fork length (mm)
3.03±0.01	55.12±0.6	3.21±0.03	55.01±0.4	24.03±1.6	121.26±0.8	31.22±1.2	136.32±0.7
4.15±0.01	58.63±0.7	5.44±0.02	56.19±0.5	26.81±1.5	125.12±0.7	41.54±1.4	151.17±0.9
6.13±0.04	76.41±0.9	7.83±0.03	78.29±0.6	30.12±1.1	131.53±0.9	62.59±1.6	159.69±1.1
8.22±0.03	91.02±1.0	11.91±0.05	86.92±0.8	33.96±1.3	136.75±1.0	87.33±1.9	174.94±1.1
11.19±0.06	94.55±1.1	17.32±0.04	88.47±0.9	38.44±1.4	138.02±1.1	114.19±1.6	192.71±1.2
15.64±0.2	117.33±1.2	29.55±0.4	91.17±1.0	43.56±1.5	140.18±1.3	144.62±1.7	217.49±1.2
21.35±0.3	121.82±1.3	44.61±0.6	102.35±1.1	50.87±1.6	155.35±1.2	179.04±1.9	235.25±1.4
27.98±0.5	124.94±1.4	63.17±0.7	115.68±1.2	61.73±1.7	163.47±1.4	230.78±2.1	250.05±1.6
35.69±0.6	150.16±1.5	89.85±0.8	120.54±1.4	90.95±1.6	180.84±1.6	310.49±2.3	283.53±1.3
44.55±0.7	159.77±1.6	122.05±0.7	125.48±1.5	123.12±1.8	201.77±1.7	400.14±2.1	308.85±1.5
55.71±0.8	189.44±1.7	158.38±0.9	131.75±1.6	145.53±1.6	210.22±2.0	510.85±2.4	325.13±1.7
72.02±0.6	197.53±1.9	210.57±0.8	156.29±2.0	163.35±1.6	220.92±2.2	645.23±2.2	337.64±1.9
88.48±0.7	215.85±1.7	258.97±1.1	160.29±1.6	177.81±1.7	228.16±2.4	730.15±2.6	358.96±2.1
103.15±0.9	230.97±1.9	309.20±1.3	171.22±1.5	191.19±1.5	230.49±2.6	801.03±2.9	374.29±2.3
115.96±0.8	255.16±2.1	371.65±1.5	178.89±1.7	203.05±1.6	235.05±2.4	865.29±3.4	384.73±2.5
123.87±0.9	274.09±2.3	485.13±1.6	180.26±1.9	213.38±1.9	242.39±2.5	913.77±3.6	395.45±2.2
131.04±1.1	283.47±2.5	573.24±1.8	184.95±1.6	222.92±2.4	248.28±2.1	955.68±3.8	399.36±2.3
138.02±1.2	294.58±2.2	648.45±2.2	188.69±1.8	230.85±2.5	250.89±2.4	993.12±4.1	402.57±2.6
144.88±1.1	310.19±2.4	709.13±2.1	191.51±1.4	238.46±2.1	252.51±2.6	1027.39±4.5	404.68±2.5
149.95±1.4	321.83±2.6	765.03±2.3	192.45±1.6	245.14±2.3	253.65±2.9	1057.58±4.8	406.82±2.8
154.08±1.3	326.75±2.8	805.36±2.4	195.36±1.8	251.28±2.5	254.97±2.7	1082.72±4.7	409.07±3.1
157.46±1.5	330.66±2.9	837.27±2.7	196.98±1.5	256.84±2.6	255.07±2.9	1103.65±5.4	411.19±3.3
160.27±1.7	334.29±3.1	858.86±3.1	198.89±1.8	241.16±2.2	256.07±3.1	1121.76±5.5	412.43±3.4
162.19±1.8	339.02±3.3	870.14±3.4	200.27±1.9	264.15±2.5	256.65±3.2	1136.83±5.7	414.12±3.5

After supplemented feed however the increase in body weight reaches its peak after 5th fortnight, which is then followed by a gradual decrease with feed supplementation (Table I). *H. molitrix* performed comparatively better with the feed supplementation compared to *Catla catla*. In *Catla catla* and *H. molitrix* the weight gain at end of study, was 436.53% and 330.37%, respectively when fed on plant supplemented feed..

The experiment was started with the initial average fork lengths of 53.23±0.05 and 124.18±0.09 mm of *Catla catla* and *H. molitrix,* respectively (Table I). At the end of experiment, *Catla catla* and *H. molitrix* attained the average total lengths of 339.02±1.08 and 414.12±2.96 mm; 200.27±1.38 and 265.65±2.32 mm in treated and control ponds, respectively (Table I). The maximum increase in total length in *Catla catla* was recorded during the 11th fortnight as 29.67 closely followed by 25.22 mm during 9th fortnights, respectively. As regards *H. molitrix* it showed a maximum increase in total lengths in the ponds supplied with artificial feed 33.49 mm during 9th followed by 25.31 mm during the 10th, respectively. 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 temperature limits of 30 to 33.73°C. While during the present study, best growth of carps was achieved within a temperature range of 24.85-31.16°C. The present results do not support the findings of Barthelmes and Jahnichen (1978), who reported the growth of silver carp (*Hypophthalmichthvs molitrix*) at water temperatures ranging from 18 to 22°C. It is evident from the above results that growth is dependent upon food consumption and surplus energy after meeting the energy requirements of basal



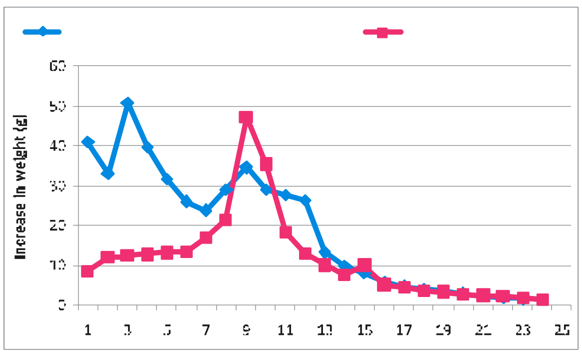


Fig. 1. Fortnightly increase (%) in wet weight of *Catla catla* (A) and *Hypophthalmicthys molitrix* (B) after feeding supplemented diet.

metabolism and activity of the fish. Feeding activity and food requirements and consumption patterns are greatly influenced by temperature. In the present study warmer months seemed to be the best period for attainment of maximum weight of the fish. It appears net efficiency was more at these temperatures.Varghese *et al*. (1980) reported that silver carp (*Hypophthalmicthys molitrix*), grass carp (*Ctenopharyngodon Idella*) male common carp (*Cyprinus Carpio*), rohu (*Labeo rohita*) and mrigal (*Cirrhina mrigala*), responded to artificial feed comprising 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 although both *Catla catla* and the *H. molitrix* is surface feeder, but latter performed better on supplementary feed under composite culture which contradicted the results of Chakarabarty *et al.* (1976).

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 an 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.

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 equations indicate that both the fish species did not deviate from the general trend of such relationship for feed supplementation. 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.

Table II.- Fork Length –Weight relationship for *Catla catla* and *H. molitrix* in feed supplemented and control pond.

Fish species	Treatment	Regression equation	r
Catla catla	Feed Supplementation	log W= -3.86+2.629 log F.L (0.231)	0.925
	Control	log W= -4.99+3.134 log F.L (0.068)	0.995
H. molitrix	Feed Supplementation	log W= -5.13+3.123 log F. L (0.041)	0.998
	Control	log W= -5.22+3.169 log F. L (0.035)	0.999

After one year experimental period all the ponds were harvested for final fish yield. Total production for *Catla catla* with feed supplementation and without any additives (control) ponds were calculated 6,961.1 2 and 1,135.33 g, respectively. The total production of 23,873.43 and 5,283.00 g were recorded in treated and control ponds, respectively for *H. molitrix*. 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 meals and concentrates on the growth of rainbow trout have been reported by various workers (Gatlin *et al.,* 2007; Lee *et al.,* 2006; Lim *et al.,* 2008; Mahboob and Sheri, 1997; Ayse, 2011). Finding alternative protein sources to replace fish meal in fish feed is important if the growth of the aquaculture industry is to be sustained (Francis *et al.,* 2001; Tacon, 1993). It has been observed in this study that both the fishes performed significantly better in terms of increase in the weight and length compared to control. The better conversion efficiency of fish feed into fish flesh in *Catla catla* and *H. molitrix* is due to their feed habit. Both the fish species are surface feeder (Mahboob and Sheri, 2002).

CONCLUSIONS

The results from the current study exhibit that *Catla catla* and *H. molitrix* have the potential for fast growth with plant based diet and may substantially enhance the growth. Both the fish species preferred to feed on the surface. *H. molitrix* performed better as compared to *Catla catla*. The findings of the present study suggest that fish meal can be replaced easily with alternative plant protein sources like cottonseed meal, maize gluten meal and sesame oil cake.

ACKNOWLEDGEMENTS:

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this Research group No. RG 1435-012.

REFRENCES

ALAM, A. K., MAUGHAN, E. AND MATTER, W. J., 1996. Growth response of indigenous and exotic carp species to different protein sources in pelleted feeds. *Aquacult. Res*., **27**: 673-679.

AYSE, G. H., 2011. The influence of replacing fish meal partially in diet with soybean meal and full fat soya on growth and body composition of rainbow trout (*Oncorhynchus mykiss*). *Pakistan J. Zool*., **43**:175-182.

BARTHELMES, D. AND JAHNICHEN, H., 1978. Diet selection and feed intake of silver carp of 3 or 4 summers. *Z. Binnenfisch. DDR,* ***25***: 331-334.

BARROWS, F.T., GAYLORD, T.G., SEALEY, W.M., PORTER, L., Smith, C.E., 2010. Supplementation of plant-based diets for rainbow trout, *Oncorhynchus mykiss* with macro-minerals and inositol. *Aquacult. Nutr.*, **16:**654–661.

BHOSALE, S.V., BHILAVE, M.P. AND NADAF, S.B., 2010. Formulation of fish feed using ingredients from plant sources. *Res. J. agric. Sci.,* **1**: 284-287

CHAKRABARTY, P.C., BASAK, P.K., SARKAR, G. AND GHOSH, G., 1979. Use of a new artificial feed in carp seed raising. In: *Symposium on inland aquaculture*, February 12, 1979 (Abs), p.110, Brackpore.

CHENG, Z.J., HARDY, R.W., USRY, J.L., 2003. Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (*Oncorhynchus mykiss*) and apparent digestibility coefficients of nutrients. *Aquaculture,* **215**:255–265.

FAO, 2010. *The state of world fisheries and aquaculture*. Rome, 218 p.

FRANCIS, G., MAKKAR, H. P. S. AND BECKER, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* **199:**197-227.

GATLIN III, D.M., BARROWS, F.T., BROWN, P.,

DABROWSKI, K., GAYLORD, T.G., HARDY, R.W., HERMAN, E., HU, G., KROGDAHL, Å., NELSON, R., OVERTURF, K., RUST, M., SEALEY, W., SKONBERG, D., SOUZA, E.J., STONE, D.,WILSON, R.,WURZTEL, E., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.,* **38**: 551–579.

GLENCROSS, B.D., CARTER, C.G., DUIJSTER, N., EVANS, D.R., DODS, K., McCAFFERTY, P., HAWKINS, W.E., MAAS, R. AND SIPSAS, S., 2004. A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **237**: 333–346.

HIDALGO, M. C., SANZ, A., GALLEGO, M. G., SURAEZ, M.D. AND HIGERA, M., 1993. Feeding of Eurpeon eel, *Anguilla anguilla*. 1. Influence of dietary carbohydrate level. *Comp. Biochem. Physiol*., **105**: 165-169.

HIGGS, D. A., DOSANJI, B. S., PRENDERGAST, A. F., BEAMS, R. M., HARDY, R. W., RILEY, W. AND DEACON, G., 1995. *Nutrition and utilization technology in aquaculture Champaignll*. Chapman and Hall Company. New York. USA, pp130-156

HORA, S.L. AND PILLAY, T. V. R., 1962. Handbook on Fish Culture in the Indo-Pacific region. FAO Fish Biol, (14): 204p.

JAVED, M., 1988. *Growth performance and meat quality of major carps as influenced by pond fertilization and feed supplementation.* Ph.D. thesis, Agric. Univ., Faisalabad.

LEE, K.J., RINCHARD, J., DABROWSKI, K., BABIAK, I., OTTOBRE, J.S., CHRISTENSEN, J.F., 2006. Long term effects of dietary cottonseed meal on growth and reproduction performance of rainbow trout: three-year study. *Anim. Feed Sci Tech*., **126**: 93–106.

LeCREN, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). *J. Anim. Ecol*., **20**:201-219.

LIM, C., WEBSTER, C.D., LEE, C.-S. (Eds.), 2008. *Alternative protein sources in aquaculture diets*. The Haworth Press, New York, NY, pp. 281-312.

MAHBOOB, S. AND SHERI, A. N., 1997. Growth performance of major, common and some Chinese carps as influenced by pond fertilization and feed supplementation in composite culture system. *J. Aquacult. Trop.,* **12**: 201-207.

MAHBOOB, S. AND SHERI, A. N., 2002. Influence of fertilizers and artificial feed on the total dressing losses, meat: bone ratios, specific gravity and density of fish and regression studies of dry weight of planktonic biomass on physico-chemical parameters. *J. Aquacult.Trop.,* **17**: 43-58.

NAYLOR, R., HARDY, R., BUREAU, D., CHIU, A., ELLIOTT, M., FARRELL, A., FORSTER, I., GATLIN, D., GOLDBERG, R., HUA, K. AND NICHOLS, P., 2009. Feeding aquaculture in an era of finite resources. *Proc. natl. Acad. Sci. U.S.A.,* **106**l: 15103–15110

TACON, A. G. J., 1993. *Feed ingredients for warmwater fish, fish meal and other processed feedstuffs*, FAO, Fish. Circ. No. 856, FAO, Rome, Italy, pp. 64.

VARGHESE, T.J., SINGIT, G.S. KESHAVANATH, P., REDDY, P.K. AND VASUDEVAPPA, C., 1980. Composite fish culture a case study. *Mysore J. agric. Sci*., **14**: 232-236.

(Received 15 January 2014, revised 8 April 2014)