

Effect of Varying Levels of Protein from Different Animal Sources on Growth and Survival of Carp, *Cirrhinus mrigala*, Reared in Cemented Cisterns

Rehana Iqbal,^{1*} Muhammad Ali,² Naeem Tariq Narejo³ and Kashif Umer¹

¹Institute of Pure and Applied Biology, Bahauddin Zakariya, University, Multan

²Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya, University, Multan.

³Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro.

Abstract.- A study was undertaken to determine the effect of protein from different animal sources on the growth and body composition of *Cirrhinus mrigala*. A total of 210 *Cirrhinus mrigala* fingerlings were distributed in completely randomized way into six treatments with three replicates each. Six experimental protein diets of varying protein level were formulated utilizing fish and chicken waste viz., fish as protein source like fishmeal with 30% protein (FM30), fishmeal with 35% protein (FM35) and fishmeal with 40% protein (FM40). Similarly chicken waste as protein source like chicken meal with 30% protein (CW30), chicken meal with 35% protein (CW35) and chicken meal with 40% protein (CW40). Controlled group diet contained a balanced mixture of proteins from both animal sources. The daily feed ration was 8% body weight of fish. Highly significant ($P < 0.001$) results were observed with respect to growth and body composition parameters. Chicken waste feeds showed better BWG, SGR, FER and PER over fish meal. With respect to body composition, chicken waste group showed higher %water content than other feeding group. Fat content was higher in FM40. In the same way protein content was higher in control group which was not also significantly ($p > 0.05$) different from FM30, FM35 FM40 and CW40. In conclusion, CW30 showed better growth performance of *C. mrigala* reared in cemented cisterns.

Key words: *C. mrigala*, fishmeal, chicken waste, growth parameters and body composition.

INTRODUCTION

Aquaculture continues to be the fastest growing animal food-producing sector and to outpace population growth with per capita supply from aquaculture increasing from 0.7 kg in 1970 to 7.8 kg in 2006, an average annual growth rate of 6.9% (FAO, 2006). It is set to overtake capture fisheries as a source of food fish. From a production of less than 1 million tons per year in the early 1950s, production in 2006 was reported to be 51.7 million tones with a value of US\$78.8billion, representing an annual growth rate of nearly 7 percent (FAO, 2008). World aquaculture is heavily dominated by the Asia-Pacific region, which accounts for 89 percent of production in terms of quantity and 77% in terms of value. This dominance is mainly due to China's enormous production, which accounts for 67 percent of global production in terms of quantity and 49% of global value. China

produces 77% of all carps (cyprinids) and 82% of the global supply of oysters (FAO, 2008).

Indian major carps, the prime cultivable freshwater fishes, are raised on simple dietary combination of plant and animal feed stuffs in India. These fishes are fast growing, attaining a marketable size of 800-1000g in less than a year and are generally propagated on extensive and or intensive scale of polyculture system (Jhingran, 1991). Among these carps, *C. mrigala* is a detritus eater with narrow range in fruit variety and is a bottom feeder subsisting mainly on decaying vegetation. It is fast growing fish and is used as component of polyculture with other species of major carps. Optimization of feeding rate of culture fish is important to achieve efficient production (Tacon and Cowey, 1985). The quantity and quality of feed consumed have pronounced effect on growth rate, efficiency of feed conversion and chemical composition (Pickering, 1993).

The protein allowances in fish diets are appreciably higher than those in the diets of terrestrial warm-blooded animals (NRC, 1993). The methods used to determine protein requirements,

* Corresponding author: hani155@yahoo.com

0030-9923/2014/0006-1599 \$ 8.00/0

Copyright 2014 Zoological Society of Pakistan

however, may overestimate requirements, in that excess dietary protein or amino acids, which cannot be stored, are catabolized preferentially over carbohydrates and fats and used for energy by some fishes (Wilson, 1989). In addition, adequate consideration has not always been given to factors such as concentration of dietary energy in the diet, amino acid composition of the dietary protein, and digestibility of the dietary protein (Wilson, 1989). Understanding the nutritional constraints and limitations used in arriving at these reported a protein requirement is important for their proper application. Protein requirements, as a proportion of the diet, decrease as fish approach maturity. For example, 25 percent protein was adequate in the diet of channel catfish of 114 to 500 g, but 35 percent protein produced faster gains than did 25 percent protein in 14- 100 g fish (Andrews *et al.*, 1980).

Various oilseed cakes and meals are produced in different parts of the world on a large scale as by-products of the edible oil industry. These include linseed, soybean, cottonseed and sunflower meal. These oil cakes and meals are fairly rich in protein and are traditionally used as feeds for farm animals. In recent years, the intensification of tilapia fry production has made it essential to develop complete and supplemental diets for use in hatcheries and nursery ponds. The efficiency of the various alternative protein sources as partial or complete dietary replacement for fishmeal has been evaluated in fish diets, *e.g.*, poultry by-product (Hernandez *et al.*, 2010), mixture of meat and bone meal, poultry by-product meal, blood meal and corn gluten meal (Ye *et al.*, 2011), canola meal (Umer and Ali, 2009), sunflower (El-Saidy and Gaber, 2002a), soybean (El-Saidy and Gaber, 20002b) and linseed meal (El-Saidy and Gaber, 2001). Soybeans, cottonseed, sunflower and linseed meals, commonly incorporated in practical fish feeds (El-Sayed, 1999), have been studied individually as plant protein replacements for fishmeal. These plant by-product meals have high protein levels and favorable essential amino acid profiles, but they are deficient in one or more essential amino acids (NRC, 1993). Therefore, it is important to study the nutritional value of combinations of plant proteins to replace fishmeal in commercial fish diets without compromising the growth and feed efficiency. The

purpose of the study was to evaluate the growth, feed utilization and whole-body composition of *C. mrigala* using various animal sources of protein.

MATERIALS AND METHODS

Experimental diets

Six experimental diets and one controlled diet were prepared to determine the growth performance and body composition of *C. mrigala*. Two animal protein sources with different levels were used. The animal proteins were taken from fish meal and chicken wastes with different levels of 30%, 35% and 40%, respectively. Controlled group diet contained a balanced mixture of proteins from both animal sources. Ingredients and proximate composition of the all experimental diets are presented in Table I.

Experimental design and feeding trial

Experiments were laid out in randomized complete block design and were conducted in cemented cisterns (size 2.5m²), Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. Fish samples of same age group 1.5±0.65 g were collected from local fishery. All the fishes were transferred to experimental laboratory in oxygen-filled polythene bags. The fries were acclimatized to experimental conditions for one week before they were removed from the reservoir tanks and transferred to cemented cisterns with circulating water system. Each cistern consisted of 10 fingerlings with a temperature 28°C, pH 7-8 and dissolved oxygen level of 5-9 were maintained throughout the experiments. To keep the survival rate high, weekly monitoring of temperature, dissolved oxygen, electric conductivity (EC) and pH was carried out. If there was any variation observed in temperature, the water circulation was increased, which could bring down or increase the temperature to a desired level (AOAC, 1984). Similarly if pH was changed to an acid side, then lime was added into the cistern to bring the pH to a range of 7-8. If temperature and pH is maintained at a required level then EC and dissolved oxygen can be controlled (AOAC, 1984). Fish were fed twice a day by hand at 8% of body weight (Khan *et al.*, 2004) in two equal portions for 90 days. Fish feeds were adjusted

Table I.- Feed formulation and proximate composition of diets applied in present study.

Ingredients (gm/kg)	Fishmeal			Chicken waste		
	30%	35%	40%	30%	35%	40%
Chicken wastes	-	-	-	400	500	600
Mustard oil cake	-	-	-	300	200	200
Rice polish	300	-	300	200	250	120
Wheat flour	90	90	90	90	40	70
Fishmeal	300	350	400	-	-	-
Wheat bran	300	150	200	-	-	-
Rice bran	-	400	-	-	-	-
Vitamins	05	05	05	05	05	05
Minerals	05	05	05	05	05	05
Total	1000	1000	1000	1000	1000	1000

* Control diet was prepared by mixing all the above feeds in equal quantity.

Table II.- Mean values and standard deviation (Parenthesis) of growth parameters of *C. mrigala* for different feeding groups.

Growth parameters	Fishmeal			Chicken waste		
	30%	35%	40%	30%	35%	40%
Body weight gain ¹	888.5±8.67	1045.3±10.6	877.8±7.46	1253.3±11.1	1244.8±11.5	1018.6±12.3
Specific growth rate ²	2.5±0.00	2.6±0.01	2.5±0.00	2.8±0.00	2.8±0.00	2.6±0.00
Feed efficiency ratio ³	1.8±0.00	2.2±0.01	1.8±0.00	2.8±0.00	2.8±0.00	2.3±0.02
Protein efficiency ratio ⁴	0.10±0.00	0.11±0.00	0.07±0.00	0.15±0.00	0.13±0.00	0.09±0.00
Feed conversion ratio ⁵	55.9±0.24	45.4±0.22	54.5±0.23	36.4±0.20	36.2±0.23	47.2±0.54
Condition factor ⁶	0.85±0.00	0.93±0.00	0.87±0.00	0.87±0.00	0.95±0.00	0.96±0.00

1, Body weight gain (%) = (Final weight – initial weight) × 100 / initial weight; 2, Specific growth rate (% day⁻¹) = (ln final weight – ln initial weight) × 100 / days; 3, Feed efficiency ratio = (Final weight – initial weight) / Total dry feed intake × 100; 4, Protein efficiency ratio (g) = Final weight – Initial weight / protein intake; 5, Feed conversion ratio = Total feed intake / weight gain; 6, Condition factor = Final weight / Final length³ × 100.

after each fifteen days by randomly weighing one fish from each group.

Growth performance

To determine the growth performance, various parameters were calculated such as fish body weight gain (BWG), feed conversion ratio (FCR), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) according to Hengswat *et al.* (1997).

Statistical analysis

The data were analyzed by ANOVA using Minitab-16. LSD was determined using Tukey's method (Zar, 1984)

RESULTS

Highly significant (P<0.001) results were observed with respect to growth and body composition parameters. Chicken waste feeds showed better BWG, SGR, FER and PER over fish meal. CW30 showed maximum growth while control showed poor growth rate. BWG was higher in CW30 which was not significantly (p>0.05) different from all other feeding groups except control group. Similar relation was observed with reference to SGR. FER was highest in CW30 which was not significantly (p>0.05) different from other chicken waste feeding groups. FCR and CF was highest in control which was significantly higher than all other feeding groups.

Table III.- Mean values and standard deviation (Parenthesis) of various body constituents and condition factor of *C. mrigala* different feeding groups.

Growth parameters	1			2		
	Fishmeal			Chicken waste		
	30%	35%	40%	30%	35%	40%
Water (%)	85.1±0.05	83.7±0.06	82.1±0.1	87.1±0.07	87.3±0.06	83.7±0.11
Dry weight (%)	14.8±0.05	16.2±0.06	17.9±0.10	12.9±0.09	12.7±0.05	16.3±0.11
Ash (wet weight) (%)	1.8±0.00	2.8±0.01	2.8±0.02	1.2±0.00	1.7±0.00	1.8±0.01
Fat (wet weight) (%)	3.27±0.00	3.94±0.00	4.70±0.04	2.49±0.00	2.83±0.01	4.38±0.04
Protein (wet weight) (%)	11.34±0.04	12.10±0.06	12.45±0.09	10.33±0.07	9.71±0.04	11.76±0.07

%dry weight of fish = dry weight of fish/wet weight of fish x 100

With respect to body composition, chicken waste group showed higher %water than other feeding group. Fat content was higher in FM40 which was not significantly ($p>0.05$) different from control, FM35 and CW40. In the same way protein content was higher in control group which was not also significantly ($p>0.05$) different from FM30, FM35 FM40 and CW40. All the results of growth performance of experimental fish and proximate analysis are shown in table 2 and 3, respectively.

DISCUSSION

Poultry waste is a rendered product obtained from the waste of poultry production and processing plants. It is usually made from inedible portions of poultry, excluding feathers. It has been studied as a FM replacement in the diets for gilthead seabream *Sparus aurata* (Alexis, 1997). Early studies had shown that chicken waste, if used alone could generally replace not more than 50% of FM protein, or growth was compromised (Fowler, 1991; Steffens, 1994). If supplemented with amino acids (lysine, methionine or tryptophan) or combined with other protein, chicken waste could show a more pronounced nutritional potential (Webster *et al.*, 1999). Our study did not agree with previous study, that difference might be due to source variation. High-quality chicken waste now contains about 70% crude protein and relatively low ash content (Nengas *et al.*, 1999; Davis and Arnold, 2000; Kureshy *et al.*, 2000), and can even be used without supplementation, replacing 75% or even 100% of the FM without a significant depression in fish performance (Alexis, 1997; Nengas *et al.*, 1999;

Takagi *et al.*, 2000). Davis and Arnold (2000) reported that replacement of 80% FM protein in practical diets for *L. vannamei* resulted in a significant increase in weight gain and FER. These varying results may relate to the species tested, but are more likely to be a consequence of the different quality of CW production, which varies among producers (Bureau *et al.*, 1999). Dong *et al.* (1993) found that there were significant differences in proximate composition and protein digestibility in CW samples obtained from six different manufacturers. Our results showed that no change with changing the source of protein in diet with respect to BWG and SGR. But highly significant variations were observed in case of FER PER. PW showed better results replacing FM. These results are comparable with values obtained for gilthead seabream fed diet containing CW, a high-fat CW available on the Greek market but higher or lower than those of gilthead seabream fed other diets containing different CW (Nengas *et al.*, 1999). Similar results were also reported by Fowler (1991), Steffens (1994) and Yang *et al.* (2004), showing that CW generally could efficiently substitute up to half of the FM protein in aquatic diets. Turker *et al.* (2005) reported that replacing FM protein by PW up to 250 g/kg in Black Sea turbot diets did not affect fish growth in winter conditions at temperatures ranging 6–8°C. Our findings are in agreement with those reported in other studies, in terms of the possible use of CW as a partial substitute for FM in *C. mrigala* diets. The results of the present study did not demonstrate significant differences between groups of *C. mrigala* fed FM30, FM35 and FM40. These findings are similar to those of El-Saidy and

Gaber (2003), who did not observe significant reduction in growth after *O. niloticus* was fed a diet in which all the fishmeal protein was substituted with that of a mixture of plant feed stuffs. All the studies reviewed above were conducted in clear-water systems, while the present study was conducted in fertilized cemented cisterns. In the latter, fish could mobilize extra dietary nutrients and minerals from natural food and the culture medium, thus making up for the nutrients deficit in the shrimp-free diet (Bowen, 1980). These reports clearly demonstrate that substituting FM with CW showed comparatively better results as it can act as manure can trigger the growth of micro fauna and flora in the pond enhancing the food availability for the experimental fish. Chicken waste could be a cheaper and useful source of fish meal in future for aquaculture farmers it should be investigated for other species cultured in water but further trials are required.

ACKNOWLEDGEMENTS

The authors are grateful to Chairman, Department of Fresh Water Biology and Fisheries, University of Sindh, Jamshoro for providing lab facilities and Field staff of Thata Fish hatchery for providing experimental fish. We are very thankful to the Bahauddin Zakariya University, Multan for providing financial support to conduct present research.

REFERENCES

- ALEXIS, M., 1997. Fish meal and fish oil replacers in Mediterranean marine fish diets. *Cahiers Options Mediter.*, **22**: 183-204.
- ANDREWS, J.W., MURAI, T. AND PAGE, J.W., 1980. Effects of dietary cholecalciferol and ergocalciferol on catfish. *Aquaculture*, **19**: 49-54.
- AOAC, 1984. *Official methods for analysis*, 14th edn. Association of Official Analytical Chemists, Washington, USA.
- BOWEN, S.H., 1980. Detrital non-protein amino acids are the key to rapid growth of *Tilapia* in Lake Valencia, Venezuela. *Science*, **207**: 1216-1218.
- BUREAU, D.P., HARRIS, A.M. AND CHO, C.Y., 1999. The effect of purified alcohol extracts from soy products on feed intake and growth of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **161**: 27-43.
- DAVIS, D.A. AND ARNOLD, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, **185**: 291-298.
- DONG, F.M., HARDY, R.W., HAARD, N.F., BARROWS, F.T., RASCO, B.A., FAIRGRIEVE, W.T. AND FORSTER, I.P., 1993. Chemical composition and protein digestibility of poultry by-product meals for salmonid diets. *Aquaculture*, **116**: 149-158.
- EL-SAIDY, D.M.S. AND GABER, M.M.A., 2001. Linseed meal- its successful use as a partial and complete replacement for fish meal in practical diets for Nile tilapia *Oreochromis niloticus*. In: *Proceedings of the Second International Conference on Animal Production and Health in Semi-Arid Areas*. Faculty of Environmental Agriculture Sciences, Suez Canal University, El-Arish-North Sinai, Egypt, pp. 635-643.
- EL-SAIDY, D.M.S. AND GABER, M.M.A., 2002a. Evaluation of de-hulled sunflower meal as partial and complete replacement for fish meal in Nile tilapia, *Oreochromis niloticus* (L.), diets. In: *Proceedings of the first annual scientific conference on animal and fish production* (Faculty of Agriculture, Al-Mansoura University, Egypt). September 24-25, 2002, pp.193-205.
- EL-SAIDY, D.M.S. AND GABER, M.M.A., 2002b. Complete replacement of fish meal by soybean meal with dietary L-lysine supplement for Nile tilapia *Oreochromis niloticus*, fingerlings. *J. World Aquac. Soc.*, **33**: 297-306.
- EL-SAIDY, D.M.S.D. AND GABER, M.M.A., 2003. Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquacul. Res.*, **34**: 1119-1127.
- EL-SAYED, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture*, **179**: 149-168.
- FAO, 2006. *State of world aquaculture 2006*. FAO Fisheries Technical Paper, vol. 500. FAO, Rome, pp. 134.
- FAO, 2008. FAO Fisheries Department, Fishery Information, Data and Statistics Unit. Fish stat Plus: Universal software for fishery statistical time series. Aquaculture production: quantities 1950–2006, Aquaculture production: values 1984–2006; Capture production: 1950–2006; Commodities production and trade: 1950–2006; Vers. 2.30. Food Outlook - Global Market Analysis. June 2008. FAO, Rome. 95 pp.
- FOWLER, L.G., 1991. Poultry by-product meal as a dietary protein source in fall chinook salmon diets. *Aquaculture*, **99**: 309-321.
- HENGSWAT, K., WARD, F.J. AND JARURATIHAMORN, P., 1997. The effect of stocking density on yield. Growth and mortality of African catfish (*Clarias gariepinus* Burchell 1822) cultured in cages. *Aquaculture*, **152**: 67-76.

- HERNANDEZ, C., OLVERA-NOVOA, M.A., HARDY, R.W., HERMOSILLO, A., REYES, C. AND GONZALEZ, B., 2010. Complete replacement of fish meal by porcine and poultry by-product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: digestibility and growth performance. *Aqua. Nutri.*, **16**: 44–53.
- JHINGRAN, A.G., 1991. *Performance of tilapia in Indian waters and its possible impact on the native ichthyofauna*. The IPFC Proceedings, Bogor, Indonesia. FAO Fisheries Report No. 458. FAO, Rome: 143-161.
- KHAN, M.A., AHMAD, I. AND ABID, S.F., 2004. Effect of ration size on growth, conversion efficiency and body composition of fingerling mrigal, *Cirrhinus mrigala* (Hamilton). *Aquacul.Nutr.*, **10**: 47-53.
- NENGAS, I., ALEXIS, M.N. AND DAVIES, S.J., 1999. High inclusion levels of poultry meals and related by products in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, **179**: 13-23.
- NRC, 1993. *Nutrient requirements of fish*. National academy of sciences, Washington D.C.
- PICKERING, A.D., 1993. Growth and stress in fish production. *Aquaculture*, **111**: 51-63.
- STEFFENS, W., 1994. Replacing fish meal with poultry by-product meal in diets for rainbow trout *Oncorhynchus mykiss*. *Aquaculture*, **124**: 27-34.
- TACON, G.J. AND COWEY, C.B., 1985. Protein and amino acid requirements. In: *Fishenergetics: new perspectives* (eds. P. Titler and P. Calow), Croom Helm, London, pp. 155-183.
- TAKAGI, S.T., HOSOKAWA, H., SHIMENO, S. AND UKAWA, M., 2000. Utilization of poultry by-product meal in a diet for red sea bream *Pagrus major*. *Nippon Suisan Gakkaishi*, **66**: 428-438.
- TURKER, A., YIGIT, M., ERGUN, S., KARAALI, B. AND ERTEKEN, A., 2005. Potential of poultry by-product meal as a substitute for fishmeal in diets for Black Sea turbot *Scophthalmus maeoticus*: growth and nutrient utilization in winter. *Israeli J. Aquacult.-Bamidgeh*, **57**: 49–61.
- UMER, K. AND ALI, M., 2009. Replacement of fishmeal with blend of canola meal and corn gluten meal, and an attempt to find alternate source of milk fat for rohu (*Labeo rohita*). *Pakistan J. Zool.*, **41**: 469-474.
- WEBSTER, C.D., THOMPSON, K.R., MORGAN, A.M., GRISBY, E.J. AND GANNAM, A.L., 2000. Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops M. saxatilis*). *Aquaculture*, **188**: 299–309.
- WILSON, R.P., 1989. Amino acids and protein. In: *Fish nutrition* (ed. J. Halver), Academic press, London, pp. 112-153.
- YANG, Y., XIE, S.Q., CUI, Y.B., ZHU, X.M., YANG, Y.X. AND YU, Y., 2004. Effect of replacement of fish meal by meat and bone meal and poultry by-product meal in diets on the growth and feed utilization of gibel carp, *Carassius auratus gibelio*. *Aquacult. Nutri.*, **10**:1-6.
- YE, J-D., WANG, K., LI, F-D., SUN, Y-Z. AND LIU, X-H., 2011. Incorporation of a mixture of meat and bone meal, poultry by-product meal, blood meal and corn gluten meal as a replacement for fish meal in practical diets of Pacific white shrimp *Litopenaeus vannamei* at two dietary protein levels. *Aquacult. Nutri.*, **17**: 337–347.
- ZAR, J.H., 1984. *Biostatistical analysis*, 2nd edn. Prentice-Hall International, Englewood Cliffs, NJ, USA

(Received 8 May 2014, revised 14 June 2014)