Preprint of paper published in: *Journal of Bioresources 3(1); 2016; 30-34.*

Protein requirement of angel fish *Pterophyllum scalare* (Schultze 1823) (Perciformes: Cichlidae).

I. K. Pai* and Maryem Shaikh Altaf
Department of Zoology, Goa University, Goa-403 206

ABSTRACT

Since time immemorial, fishery, which helps in food security and poverty alleviation, has been one of the oldest professions of man and remained to be so, even today. Further, ornamental fisheries has also provides ample scope for improving economy and employment generation. Today, Fish culturing is a major part of aquaculture. Like in other animals, in fishes too, food determines various physiological, developmental or even reproductive aspects. So, several attempts have been made by various wo[1]rkers, to alter food and other environmental conditions to determine optimum growth. However, as there are hardly any attempts to analyze suitable protein requirements for having optimum health and development in fish diet, Thus, an attempt has been made to determine the optimum protein requirement for the health growth and development in one of the popular aquarium angel fishes, P*terophyllus scalare* by altering protein and lipid contents from 30%-40% protein and 6% -10% lipids, in their feed. The parameters such as body weight gain, food conversion ratio (FCR), specific growth rate (SGR), protein efficiency rate (PER), were analyzed. The studies indicate that, the maximum weight gain was at 35% Protein + 6% Lipid (1.99 ± 0.04gm), maximum FCR was 1.71 ± 0.03%) at 40% P + 10% L; SGR and PER was maximum at 30% P + 6% L was 2.60 ± 0.06% and 2.33 ± 0.04%), suggesting that, food with 30% protein and 6% Lipid is ideal for angel fish for its optimum growth.

Key words: Angel fish *Pterophyllum scalare,* fish food, protein requirement

INTRODUCTION

Among the fishes used in freshwater ornamental fisheries, the majority (> 90%) are bred in captivity, compared to only about 25 of a total of 8000 species in case of marine fishes. FAO (2016). The ornamental fish industry relies heavily on the export and import of introduced species (FAO, 2016). In India, like other countries, most popular aquarium fishes used in ornamental fisheries, due to their attractive coloration, sturdiness, stability, ability to withstand

1 **Corresponding author. Email: ikpai@unigoa.ac.in*

considerable environmental variations, relatively easy maintenance etc., are angel fishes, guppy's, swordtails, platys and molly's.

Production of aquaculture species, in semi intensive pond culture system, demands the use of artificial feed, as a supplementary source of nutrition. Using of such artificial diet for fishes can be traced as early as 1927, by Ida Melon. Akiyama *et al*., (1991), Tacon (1993) have reported that the most commonly used animal protein sources in the diet are fish meal, whose level could range between 10-50% of the operational costs. Due to increasing cost and short supply of fish meal, cost of fish feed has increased drastically over the years (Higuera and Gardenete, 1989; McCoy, 1990; Bimbo and Crowtber, 1992). So, people have started concentrating on low cost fish feed, by using plant protein sources (Tacon and Jackson, 1985) such as soybean (Dabrowski *et al*., 1989), which seem to be more promising, as a substitute, as they are almost half the price of fish meal. But, the inclination to use substitute plant and animal proteins, as a low cost substitute for fish meal, such alternatives are known to have lower nutritional value, which will result in lower growth rates or a reduced performance of the cultured animals. In addition, such protein sources may cause slight to severe effects on nutritional status of an animal. Despite the above, throughout the world, the search for alternative low cost substitutes is in vogue (Tacon *et al*., 1983; Stafford and Tacon, 1985; Tacon, and Jackson, 1985; Pongmaneerat and Watanabe, 1991; Rumsey, 1993).

By partly replacing dietary proteins by lipids, protein retention in several fish species could be improved. Such protein sparing effects have been experimentally proved in salmon (Garcia *et. al*., 1981; Johnson *et al*., 1991), trout (Beamish and Mediandn, 1986), carp (Watanabe, 1987), hybrid striped bass (Nematipour *et al.,* 1992), yellowtail (Shimeno *et al*., 1979), red sea bream (Takeuschi *et al.,* 1991). Cho and Bureau (2001) reported that, improving digestibility of diet formulation and optimizing feeding regimens can improve feed utilization efficiency in farmed fishes.

It is well known that, fish meal has essential amino acid, fatty acids, highly digestible, low carbohydrates etc., Cowey and Sargent (1977) reported that lipid is known to be one of the important nutrients next to protein, which plays a major role in optimum utilization of dietary protein for growth. Lipids are almost completely digestible by fish and seem to be favored over carbohydrates as an energy source. Fishes are also known for utilizing protein preferentially over lipid or carbohydrate as an energy source. Therefore, it is important from nutritional, environmental and economic point of view to optimize the ration of protein and lipids levels.

In view of the above backdrop, this experiment was conducted to determine the optimum protein and lipid percentage in fish feed, with angel fish as an experimental organism.

MATERIALS AND METHODS

One of the most popular aquarium fish, Angel fish (P*terophyllum scalare*) was utilized in the present experiment. Uniformly sized, healthy fish fingerlings were procured from the freshwater ornamental fish hatchery of the Indian Council of Agricultural Research (ICAR) complex, Old Goa, India. The fishes were given pre-acclimatization treatment by treating with 0.05% potassium permanganate solution, for two minutes, to make them free from external parasites and pathogens, if any present. Before initiating the feeding trials, they were kept in 1.0 X 1.0 X 1.0 m^3 cement tanks provided with aerator for the survival of the experimental animals. These tanks were filled with 70L of freshwater, for acclimatization to the laboratory condition. During this period, they were fed 5% of their body weight divided in to two feeds daily, with locally available commercial diet (Jalaram's Fish Feed (Premium).

During the experimental period, the fishes were fed separately with nine formulations of food having 30% Protein (P) + 6% Lipid (L); 30% P + 8% L; 30% P +10% L; 35% P + 6% L; 35% P + 8% L; 35% P + 10% L; 40% P + 6% L; 40% P + 8% L and 40% P + 10% L. Three replicated with 10 fishes each in 1m x1m x 1m size with 50L were maintained with water quality as mentioned in Table-1. All the batches were fed twice daily, with the experimental diet. Further, Food conversion Ratio (FCR), Specific Growth Rate (SGR) and Protein Efficiency Rate (PER) was calculated by following Gerking (1971), Brown (1957) and Donald (1976) methods respectively.

Food conversion Ratio (FCR) = G x 100

R

G=Weight gain (gm)

R= amount of food consumed by fish (gm)

$$\text{Specific Growth Rate (SGR)} = \frac{\text{LnW2-LnW1}}{T} \times 100 \ldots \text{\% gm/day}$$

Ln W1= Ln of initial weight of fishes

Ln W2= Ln of final weight of fishes

T= days of experiment (70 days)

$$\text{Protein Efficiency Rate (PER)} = \frac{G}{F} \text{ gm/days}$$

G= amount of consumed protein by fish

F= Weight gain (gm)

The data obtained thereby was subjected to statistical analyses like ANOVA and the comparison among the groups, was done by Duncan multiple range test at P>0.05.

RESULTS

Table-1 provides the data on, the quality of water used for the experiment. The water quality was tested by following procedure as prescribed in APHA (1985). The result indicate that, the temperature was between 26.0-30.0 ^{0}C, pH was 6.5-7.2; DO was 6.72-7.23 mg/l; Hardness recorded was ranging from 93.0-106.2 mg/l; alkalinity raged between 94.0-108.3 mg/l; Nitrate was present at a range between 12.0-20.0 mg/l; while nitrite was varied from 0.04-0.09 mg/l, indicating the quality of water is very well within the permissible level.

Table-2 exhibits that, the body weight of the experimental fishes varied from 1.61 ± 0.01 to 1.67 ± 0.03gm, which is the normal weight range for the Angle fish fingerlings. The final body weight, after feeding experimental diet for 70 days, was ranging from 2.96 ± 0.11 (35% P+10%L) to 3.61 ± 0.07gm (40% P + 6% L) showing considerable improvement of the body weight. The weight gain varied from 1.36 ± 0.03 (40% P + 10% L) to 1.99 ± 0.04gm (35% P + 6% L). FCR showed a range between 1.44 ± 0.02 (30% P + 6% L) and 1.71 ± 0.03% (40% P + 10% L). SGR range was between 2.00 ± 0.05 (40% P + 10% L) and 2.66 ± 0.04 % (35% P + 6% L). PER was in the range of 1.47 ± 0.02 (40% P + 10% L) and 2.33 ± 0.04 (30% P + 6% L). The results reveal that, at 30% P + 6% L feed formulation, PER ratio was the maximum, indicating reaching of optimum protein efficiency by the fish. Further, the feed with 35% P+ 6% L recorded for highest total weight gain and SGR, demonstrating the optimum weight gain and specific growth rate in angel fishes. Best feed conversion ratio of 1.71 ± 0.03 was seen when the fingerlings were fed with 40% P+10%L feed, signifying the optimum conversion of feed to nutrition.

The experiments indicate that feed with 35% P+ 6% L best for angel fish fingerlings during their early growth stage.

DISCUSSION

Cultured fish require protein, lipids, vitamins and minerals in their diet for growth, reproduction, and other normal physiological functions. But most of the works on protein analyses has concentrated upon juvenile fish or upon rapidly growing young market fish as these have high protein dietary requirements (Wilson, 1986). Protein is the main constituent of the fish body thus sufficient dietary supply is needed for optimum growth. Protein is the most expensive macronutrient in fish diet (Pillay, 1990). So, the amount of protein in the diet should be just enough for fish growth, where the excess protein in fish diets may be wasteful and cause diets to be unnecessarily expensive (Ahmad, 2000). Reducing feed costs could be a key factor for successful development of aquaculture. The dietary protein are always considered as of paramount importance in fish feeding (Bahnasawy, 2009) and the requirement of the same for fish fry is high and ranges from 35% to 56% (Jauncy and Ross, 1982). Furthermore, Wilson and Halver (1986), Wilson (1989), Pillay (1990) and El-Sayed and Teshima (1991) found that, dietary protein requirements decreased with increasing fish size and age. Keeping in view of the above studies, the present work was undertaken on one of the popular aquarium Angel fish *Pterophyllum scalare.*

Though the Fish do not have a true protein requirement but require a balanced combination of the 20 major essential and nonessential amino acids that make up proteins. Fish utilize dietary proteins by digesting them into free amino acids, which are absorbed into the blood and distributed to tissues throughout the body where they are then reconstituted into new specific proteins of the fish tissues.

Dabrowski (1979) reported different patterns of changes in PER in relation to dietary protein level and found that, the relationship between dietary protein and PER differs from species to species. In the present studies also PER was analyzed and the results obtained, is in agreement with Dobrowski (1979) observations.

In the present studies, the feed with 35% P+ 6% L was proved to be the best, for angel fish fingerlings, during their early growth stage. Which is in agreement with studies of Ahmad (2000), who also reported that, diets containing 35% protein is recommended for fingerlings Nile tilapia *Oreochromis niloticus* L (~0.5 g) and adult (grow out) fish (20-40 g).

Acknowledgement: The authors are thankful to Dr. K.N.Mohanta, Fishery field Laboratory, ICAR Complex, Old Goa, Goa, India, for his support, encouragement and laboratory facilities

BIBLIOGRAPHY:

- Ahmad, M.H. Improve productive performance in fish. Ph.D. Dissertation, Animal prod. department, faculty of agriculture, Zagazig University (2000).
- Akiyama, D.M., W.G.Dominy and A.L.Lawrence: Penaeid shrimp nutrition. In: Marine Shrimp culture: Principles and Practices (Ed: Fast, A.W. and L.J. Lester), Elsevier Publ. Amsterdam, The Netherlands: 535-568 (1991).
- APHA: Standard methods for examination of water and waste water. 16th Edn., APHA, Washington (1985).
- Bahnasaw, M.H.:Effect of dietary protein levels on growth performance and body composition of monosex Nile Tilapia, *Oreochromis niloticus* L. reared in fertilized tanks. *Pak. Jl. of Nutri*. **8** (5): 674-678 (2009).
- Beamish, F.W.H. and T.E.Mediandn: Protein sparing effects in large rainbow trout, *Oncorhynhus mykiss, Aquaculture*, **55**: 35-42 (1986).
- Bimbo, A.R. and B. Crowtber: Fish meal and oil: Current uses, *Jl. of Am. Chem. Soc.* **69** (3): 221-227 (1992).
- Brown, M. E.: Experimental studies on growth in fish physiology (Ed: M. E. Brown,) Academic Press, New York, **1**:361-400 (1957).

- Cho, C.Y. and D.P.Bureau: A review of diet formation strategies and feeding systems to reduce excretory and feed waste in aquaculture. *Aquacul. Res*. **32** (Suppl.1): 349-360 (2001).
- Cowey, C.B. and J.R.Sargent: Lipid nutrition in fish. *Comp. Physiol*.57B:269-273 (1977).
- Dabrowski, K., P.Poczynski, G.Kock and B.Berger: Effects of partially or totally replacing fish meal protein on growth, food utilization and proteolytic enzyme activities in rainbow trout (*Salmo gairdneri*): new *in vivo* test for exocrine secretion. *Aquaculture,* **77**: 29-49 (1989).
- Dabrowski, K.: Feeding requirements of fish with particular attention to common carp, A review. Polish Arch. Hydrobiol. **26**: 135-158 (1979).
- Donald, L., J.R.Garling and R.P.Wisan: Optimum dietary protein to energy rations for channel catfish fingerlings*. J. Nutri*. **106**: 1368-1375 (1976).
- El-Sayed A.F.M. and S.Teshima: Tilapia nutrition in aquaculture. *Reviews in Aquatic Sciences,* **5**: 247-265 (1991).
- FAO: Ornamental fish, http://www.fao.org/fishery/topic/13611/en (2016)
- Garcia,M., S.Zamora and M.A.Lopez: The influence of partial replacement of protein by fat in the diet on the protein utilization by the rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol*. **68B**: 457-460 (1981).
- Gerking, S. D.: Influence of feeding and body weight in protein metabolism in blue gill sunfish. *Physio. Zool*. **44**: 9-190 (1971).
- Hancz, C.: Fish nutrition and feeding (Ch.1: Fish Nutrition) Educatio Társadalmi Szolgáltató Nonprofit Kft. Digitális Pedagógiai Osztály Rábai Cecília, H-1122, Budapest, Hungary (2011).
- Higuera, M. and G.Gardenete: Fuentes alternativas de protean y energia en acuicultura. In: Alimentacion en Acquaculture (Ed:Espinosa de los Monteros and I Lambreta): FEUGA, Madrid:59-101 (1989).
- Ida Mellen: The Natural and Artificial Foods of Fishes. *Transactions of the American Fisheries Society* Volume **57** (1): 120-142 (1927).
- Jauncey, K. and B.Ross: A guide to tilapia feed and feeding. University of Stirling, Scotland, UK. (1982).
- Johnson, F., M.Hillested and E.Austreng: High energy diets for Atlantic salmon. Effects on pollution, Fish nutrition in practice, Biarritz (France) Les Colloques, **61**:391-401 (1991)
- McCoy, H.D.: Fishmeal-the critical ingredient in aquaculture feeds. *Aquaculture magazine,* **16** (2):43-50 (1990).
- Pillay T.V. R.: Aquaculture: Principles and practices. Fishing News Book. Blackwell Scientific Publications, Ltd., Oxford, UK. pp. 575 (1990).
- Pongmaneerat, I. and T.Watanabe: Nutritive value of protein of feed ingredient for carp.*Cyprinus carpio*, *Nippon Suisan Gakkaishi*, **57**: 503-510 (1991).
- Rumsey, G.L.: Fish meal and alternate source of protein in fish feeds update, *Fisheries,* **18** (7): 14-19 (1993).
- Shimeno, S., H.Hosokawa and M.Takeda: The importance of carbohydrates in the diet of carnivores fish. *Proc. World Symp. Fin fish Nutr. Fish feed Technol*. **1**: 20-23 (1979).
- Tacon, A.G.J.: Fish ingredient for carnivorous fish species: alternatives to fishmeal and other fishery resources, FAO, *Fish Circ.* 881: pp35 (1993).

- Tacon, A.G.J. and A.J.Jackson: Utilization of conventional and unconventional protein sources in practical fish feeds. In: Nutrition and Feeding in fish (Ed: Cowey, C. B., Mackie, A. and J. Bell) Academic Press, London: 119-145. (1985).
- Tacon A.G.J., E.A.Stafford and C.A.Edwards: A preliminary investigation on the nutritive value of three terrestrial lumbricid worms for rainbow trout. *Aquaculture,* **35**: 187-199 (1983).
- Takeuschi,T., Y.Shiina and T.Watanabe: Suitable protein and lipid levels in diet for fingerlings of red sea bream *Pagrus major. Nippon Suisan Gakkaishi* 55(2):521-527 (1991).
- Nematipour, G.R., M.L.Brown and D.M.Gatlin: Effects of dietary energy: protein ratio on growth characteristics and body composition of hybrid striped bass *Morone chrysops* x *M. saxtilis. Aquaculture*, **107**: 359-368 (1992).
- Watanabe,T.P: Lipid nutrition in fish. *Comp. Biochem. Physiol*. **73**: 3-15 (1987).
- Wilson, R.P. and J.E.Halver: Protein and amino acid requirements of fishes. *Ann. Rev. Nutr*. 6: 225-244 (1986).
- Wilson, R.P.: Protein and amino acid requirements of fishes (In: S. Shiau (Ed.). *Progress in Fish Nutrition*, National Taiwan Ocean University, Keelung, Taiwan: 51-76 (1989).

Table 1: Quality of the water used for the experiment

PARAMETER	RANGE
Temperature	26.0 - 30.0 deg C
pH	6.5-7.2
Dissolved Oxygen	6.72-7.23 mg/l
Hardness (CaCO3)	93.0-106.2 mg/l
Alkalinity	94.0-108.3 mg/l
Nitrate	12.0-20.0 mg/l
Nitrite	0.04-0.09 mg/l

Table 2: Effect of protein and lipid levels on growth and nutrient utilization in angel fish *Pterophyllus scalare*

Nutrient level	Nutritional indices					
	Initial wt (g) (Mean± SD)	Final wt. (g) (Mean± SD)	Weight Gain (g) (Mean± SD)	Feed Conversion Ratio (%) (Mean± SD)	Specific Growth Rate (Mean± SD)	Protein Efficiency Rate (Mean± SD)
30% P+6% L	1.65 ± 0.03a	3.60 ± 0.04a	1.95 ±0.04a	1.44 ± 0.02	2.60 ± 0.06a	**2.33 ± 0.04a**
30% P+8% L	1.64 ± 0.02a	3.21 ± 0.06 a	1.57 ± 0.06b	1.53 ± 0.03 de	2.24 ± 0.06b	2.13 ± 0.04b
0% P+ 10%L	1.61 ± 0.01a	3.09 ± 0.06bc	1.48 ± 0.07bc	1.64 ± 0.03ab	2.17 ± 0.08bc	2.01 ± 0.03c
35% P+ 6% L	1.63 ± 0.01a	3.61 ± 0.07a	**1.99 ± 0.04a**	1.47 ± 0.03ef	**2.66 ± 0.04a**	1.94 ± 0.03c
35%P+ 8% L	1.64 ± 0.03a	3.18 ± 0.04bc	1.54 ± 0.08bc	1.56 ± 0.02cd	2.21 ± 0.06b	1.78 ± 0.04d
35% P+10%L	1.60 ± 0.00a	2.96 ± 0.11c	1.36 ± 0.10c	1.68 ± 0.03ab	2.04 ± 0.11bc	1.68 ± 0.02e
40% P+ 6%L	1.67 ± 0.03a	3.61 ± 0.11a	1.94 ± 0.07a	1.50 ± 0.03def	2.57 ± 0.05a	1.69 ± 0.02de
40% P+ 8% L	1.66 ± 0.02a	3.17 ± 0.09bc	1.50 ± 0.10bc	1.63 ± 0.02bc	2.14 ± 0.09bc	1.53 ± 0.03f
40% P+10%L	1.65 ± 0.03a	3.01 ± 0.11bc	1.36 ± 0.03bc	**1.71 ±0.03a**	2.00 ± 0.05c	1.47 ± 0.02f