Fishery Technology
2002, Vol. 39(2) pp : 88 - 93

Impact of Dietary Protein on Growth, Feed utilization and Body composition of *Puntius parrah*

R. Bindhu, N. Ramanujan and C. Sambhu
Post Graduate Department of Zoology and Industrial Fisheries
Sree Narayana College, Kollam - 691 001, Kerala, India

The juveniles of *Puntius parrah* were fed with fishmeal based protein diets at concentrations of 10%, 20%, 30%, 40% and 50% by weight for a period of 120 days in cement cisterns. Protein diet at 50% produced superior growth, maximum food consumption, high conversion efficiency and better protein digestibility. Digestive enzyme activity and RNA/DNA ratio were higher in 50% protein diet fed fish. The total dry matter and protein content of the body showed high values in the fish fed with 50% protein diet. 50% protein diet is found optimum for growth enhancement in *Puntius parrah*.

Key words: Protein diets, Feed utilization, RNA/DNA ratio, growth, body composition, *Puntius parrah*

Proteins are rapidly usable sources of calories and a high degree of digestion and absorption is expected from protein contained in the diet. Dietary protein is required for three basic functions, repletion of depleted tissues (maintenance), formation of new additional protein (growth) and gonad formation (reproduction). Several studies have been carried out to assess the nutritional requirements to increase production of cultivable fish (Islam & Hossain, 1994; Ramnarine, 1995; Sumagaysay and Borlongan, 1995; Mohanty & Sumantara, 1996, Nayak *et al.*, 1996, Bidhan, 1992).

As protein contributes significantly towards the cost of fish feed, it becomes essential to find out the optimum protein requirements for growth, survival and reproductive performance. The present study was, therefore, undertaken to assess the effect of dietary protein levels on growth, feed utilization, tissue indices, digestive enzyme activity, RNA/DNA ratio and proximate composition of *Puntius parrah.*

Materials and Methods

The growth of *P. parrah* was evaluated by culturing them in cement cisterns (5x4x1m) for a period of 120 days. Specimens of *P. parrah* were collected from the Kallada river and they were kept in cement cisterns for 10 days prior to the experiment. Healthy specimens of uniform size were randomly stocked at the rate of 50 per cement cistern and the treatment were randomly assigned to the cisterns. A fish meal based pelleted diet having different levels of protein (10%, 20%, 30%, 40% and 50% by weight) were prepared (Table 1) following the methods of Hardy (1980). The feed was administered at 10% of the body weight daily, which was split into two rations, one fed in the morning and the other in the after noon. After every fortnightly sampling, the quantity of the feed was readjusted based on the growth of the experimental fish. Fish were sampled once in every 15 days and a minimum of 30 fish were collected at random to record the total length and weight. Survival was noted and recorded daily. On the 120th day, the experiment was terminated. The tanks were completely drained and all the surviving fish were collected. Their individual length and weight were recorded. On termination of the experiment, five fish from each treatment were dissected out, the viscera, liver and gonad were taken and weighed and the body indices were worked out as follows.

$$\text{Tissue indices (\%)} = \frac{\text{Weight of tissue (g)}}{\text{weight of fish (g)}} \times 100$$

A short term experiment for 30 days was conducted in the laboratory to assess the feed consumption, assimilation, conversion efficiency, assimilation efficiency, apparent protein and lipid digestibility of the experimental fish in plastic troughs (50 l) employing 5 fish each. Each treatment was replicated three times. Initial length and weight of all fish were recorded before starting the experiment. To determine the nutrient digestibility, fish were provided with weighed amount of feed (5% of the body weight) for 6 h daily. About 90% of the water in each trough was exchanged daily causing minimum disturbance to the fish. The unconsumed feed was siphoned out 6 h after feeding. The faecal matter was siphoned out the next day prior to feeding. Unconsumed feed and the faecal matter were oven dried (60°C) and weighed. The dried faecal matter of each treatment was pooled together in separate containers and used for nutrient analysis. On termination of the experiment, the final length and weight of all the fish were recorded. They were then sacrificed, dried in the oven (60°C), weighed and kept in a desiccator for proximate analysis. Feed conversion efficiency, assimilation efficiency and nutrient digestibility were calculated (Halver, 1972) as follows.

Table 1. The Proportion of various ingredients in the diet

Ingredients	Protein in the diet				
	10%	20%	30%	40%	50%
Rice bran (%)	45.29	34.82	24.35	18.88	3.42
Tapioca flour (%)	45.29	34.82	24.35	18.88	3.42
Fishmeal (%)	4.71	15.18	25.65	36.12	46.58
Ground oilcake (%)	4.71	15.18	25.65	36.12	46.58
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition of the diet*					
Moisture (%)	12.38	12.40	12.31	12.35	12.36
Protein (%)	10.02	20.12	30.03	40.01	50.02
Lipid (%)	9.13	9.15	9.45	9.53	9.16
Carbohydrate (%)	13.35	14.12	15.21	13.20	12.63
Ash (%)	5.11	5.32	5.63	5.45	5.21
NFE (%)	51.10	38.89	27.34	19.46	10.62

* Expressed on dry weight basis; ** Nitrogen Free Extract

$$\text{Feed Conversion Efficiency} = \frac{\text{Net weight gain (g)}}{\text{Feed consumed (g)}} \times 100$$

$$\text{Apparent Nutrient Digestibility} = \frac{\text{Nutrient in feed-Nutrient in excreta}}{\text{Nutrient in feed}} \times 100$$

The influence of protein feeds on digestive enzymes (protease, amylase and lipase) activity in the foregut, midgut and hindgut of the experimental fish was studied. On termination of the experiment, five fish from each treatment were collected and used for the enzyme analysis. Saccharogenic assay was followed for estimating amylase activity (King, 1965). Unit amylase activity (AU) was calculated as mg maltose liberated during 10 min at 30°. The total protease activity was measured by the casein digestion method (Kumitz, 1947) by measuring the tyrosine content of peptides produced in15 min by proteolysis in the trichloroacetic acid supernatant. Unit protease activity (PU) was expressed as the amount of tyrosine liberated in 15 min. Bier's titrimetric method (1962) was used with minor modification for lipase activity (LU) by estimating the fatty acids liberated by the enzyme from triglyceride emulsion by titration with a standard alkali.

DNA and RNA were estimated by the methods of Giles & Myres (1965) and Ceriotti (1955). Proximate composition of experimental and control fish were analyzed on termination of the culture experiments. Five fish from each treatment were collected, minced and dried at 60°C for 12 h. The oven dried samples were powdered and stored in air tight containers for biochemical analysis (AOAC, 1990).

Analysis of variance (Snedecor & Cochran, 1968) was employed to test the statistical significance of the final mean length and weight of the fish between

treatments. Duncan's (1955) multiple range test (Steel & Torrie, 1980) was applied to find out the statistical difference between treatments.

Results and Discussion

The growth pattern in weight of *P. parrah* during the culture period is given in Fig.1. The fish fed with 10% and 20% protein diet died on the second week of the experiment. High mortality was also found in fish which received 30% protein feed. Best percentage survival was observed in 50% fish. Other groups showed more than 80% survival with 50% protein group accounting about 86% survival (Table 2). The results of the present study clearly showed the effect of dietary protein on the growth of *P. parrah*. Fish were found to be very active and grew faster in higher levels of protein diets. Treatment with low levels of protein diets (10% and 20%) was found detrimental to growth and the fish recorded complete mortality on the 15th day of the experiment. Fish which received 50% protein in their diet showed superior growth while the fish which received 30% protein showed minimum growth. Considerable work have been reported on the role of dietary protein on the growth of cultivable fish (Sindhu, 1992; Islam & Hossain, 1994; Ramnairine, 1995); Sumagaysay & Borlongan, 1995; El-Dahhar & Lowell, 1996; Gunasekera *et al.*, 1996; Mohanty & Sumantara, 1996). Similar higher growth enhancement was also reported in tilapia when fed with 40% protein in the diet (Stickney, 1972; Kiron, 1995). Sambhu (1996) reported that growth enhancement was obtained in *Etroplus suratensis* when fed with 40% protein in the diet. Sherly (1997) also reported the same results in fringed lipped carp, *Labeo fimbriatus*. Thus it can be inferred that the optimum level of protein required for the growth enhancement in *P. parrah* is 50% in the diet.

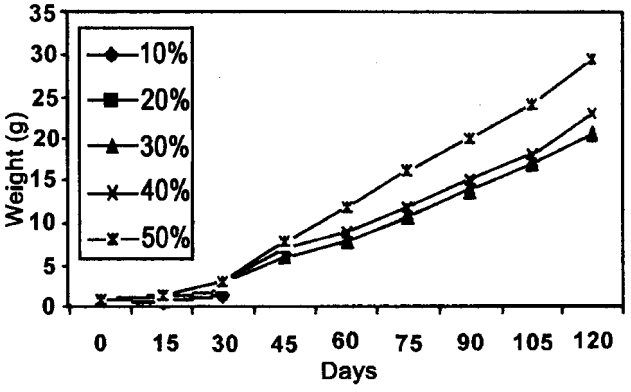

Fig. 1. Growth in weight of *P. parrah* fed protein diets

The details of feed utilization studies are shown in Table 3. The feed conversion efficiency was found to increase from 30% to 50%. High feed conversion efficiency was observed with 50% protein diet and low with 30% protein diet. The food assimilation efficiency was maximum in the fish fed with 50% protein feed and minimum with 30% protein. Fish fed with 40% and 50% protein

Table 2. Growth and survival of P. parrah fed various protein diets

Parameters	Treatments				
	10% M±SD	20% M±SD	30% M±SD	40% M±SD	50% M±SD
Initial length (cm)	2.15±0.18	2.17±0.15	2.12±0.18	2.05±0.16	2.05±0.16
Initial weight (g)	0.92±0.03	0.92±0.03	0.92±0.03	0.92±0.03	0.92±0.03
Final length (cm)	Died	Died	8.15±1.08	10.03±1.71	12.15±1.08
Final weight (g*)**			20.50±3.11[a]	23.10±2.19[b]	29.50±3.11[c]
Net weight gain (g)			19.39±1.00	22.00±2.34	28.39±3.00
S.G.R. (%)			2.02±0.10	2.15±0.11	2.32±0.14
Survival (%)			80.00±2.00	82.00±2.00	86.00±2.00

** $p<0.01$ (n=20)

a, b, c - Means with the same superscripts do not differ (Duncan's multiple range test)

Table 3. Feed utilization of *P. parrah* fed with protein diets

Parameters	Protein diets 30% M	30% SD	40% M	40% SD	50% M	50% SD
Consumption (g)*	22.07±1.89[a]		25.39±2.45[b]		27.19±1.86[c]	
Assimilation (g)	18.44±2.17		18.33±1.75		19.38±0.19	
Conversion efficiency (%)	19.98±1.99		22.52±2.74		24.92±1.74	
Protein digestibility (%)**	66.39±3.14[a]		69.39±3.41[b]		70.31±3.97[c]	
Lipid digestibility (%)	80.79±2.19		82.24±2.87		88.46±3.81	

a, b, c - Means with the same superscripts do not differ (Duncan's multiple range test)

diets grew faster than other fish by consuming more food. Similar results were recorded in Tilapia when fed 40% and 50% protein diets (Nayak *et al.*, 1996). Fish fed with higher protein diets showed better protein and lipid digestibility. The increased nutrient digestibility observed under 50% protein diet may be a reason of the enhanced growth exhibited by the fish in this treatment.

Various tissue indices such as VSI, HIS and GSI were found maximum in the fish fed with 50% protein diet (Fig.2). This indicates 50% protein in the diet is the optimum level required for the development of tissues. Similar higher tissue indices were also observed in rainbow trout and tilapia treated with 40% protein in the diet (Alexis, 1986; Kiron, 1996).

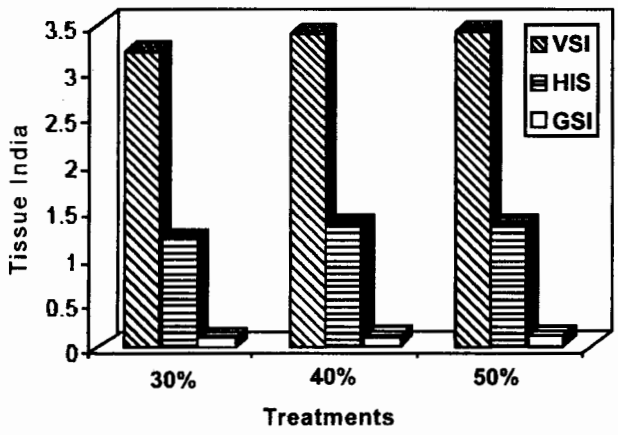

Fig. 2. Tissue indices of *P. parrah* fed protein diets

Data regarding the various digestive enzyme activities in the foregut, midgut and hindgut of *P. parrah* are presented in Table 4. Enzyme activity showed an increasing trend in fast growing fish. Highest protease activity was found in the foregut of the 50% protein fed fish and lowest in the 30% protein fed fish. Maximum amylase activity was obtained in the foregut of 50% protein fed fish, which showed highest growth in the experiment. Comparatively poor lipase activity was noticed in the alimentary canal of *P. parrah*. However, it was high in the foregut of 50% protein fed fish. Enzyme activities in the gut of fish are a function of species, feeding habit, diet, temperature, pH, age of fish and exogenous enzymes. Several studies have revealed that the relative activity of the digestive enzymes can be correlated with the nature and composition of the food consumed (Dhange, 1968; Fish, 1960). In the present experiment amylase activity was found to be high in the fish fed with 50% protein diet which showed maximum growth. Highest amylase activity was also observed in the foregut of *C. carpio* (Sindhu, 1993), tilapia (Kiron, 1995), pearlspot (Sambhu, 1996) and in fringed lipped carp (Sherly,

Table 4. Digestive enzyme activity of *P. parrah* fed various protein diets

Parameters	Protein diets 30% M	30% SD	40% M	40% SD	50% M	50% SD
Amylase						
Fore gut**	404.29±4.31[a]		464.29±3.86[b]		518.36±2.93[c]	
Mid gut	301.22±5.81		361.86±3.07		426.21±4.81[c]	
Hind gut	164.29±3.59		189.99±4.81		239.44±4.86	
Protease						
Fore gut**	401.20±2.43[a]		491.39±3.86[b]		527.33±3.44[c]	
Mid gut**	255.39±2.45[a]		400.11±3.86[b]		451.25±3.75[c]	
Hind gut	190.24±1.89		231.11±2.88		260.17±3.99	
Lipase						
Fore gut**	1.34±0.02[a]		2.93±0.12[b]		4.37±0.32[c]	
Mid gut	0.68±0.02		0.79±0.01		0.99±0.02	
Hind gut	0.23±0.07		0.39±0.01		0.22±0.01	

* Total activity = Unit enzyme activity/g tissue; ** $p<0.01$

a, b, c - Means with the same superscripts do not differ (Duncan's multiple range test)

Table 5. Nucleic acid content in the muscle of *P. parrah* fed protein diets

Parameters	Protein diets					
	30%		40%		50%	
	M	SD	M	SD	M	SD
Muscle						
RNA**	98.34±2.86[a]		112.31±0.79[b]		130.24±2.07[c]	
DNA	11.03±0.52		11.89±0.41		11.62±0.44	
RNA/DNA*	8.91±0.81[a]		9.44±0.61[b]		9.68±0.73[b]	
Liver						
RNA**	119.33±1.02[a]		132.22±1.37[b]		146.73±1.07[c]	
DNA	9.73±0.13		10.38±0.18		10.30±0.09	
RNA/DNA*	12.20±0.63[a]		12.70±0.51[b]		13.57±0.72[b]	

** $p<0.01$

a, b, c - Means with the same superscripts do not differ (Duncan's multiple range test)

1997). Protease activity was found to be generally high in the fish fed with 50% protein. Bondi & Spandrof (1954) found higher proteolytic activity of the pancreas extract in common carp when fed higher levels of protein. Kawai & Ikeda (1971) reported adaptive changes in the activity of proteolytic enzymes in *C. carpio* in relation to the type of diet.

Effect of protein on nucleic acid contents in the muscle of *P. parrah* is presented in Table 5. In the present study high RNA/DNA ratio was observed in the muscle of fish fed with 50% protein and lowest in the fish provided with 30% protein diet. Maximum RNA was found in the muscle of 50% protein diet consumed fish and it was minimum in 30% protein feed consumed fish. Bulow (1970) observed that with the increasing levels of food available to the fish, there was an increase in RNA/DNA ratio in the liver and alimentary canal with the overall increase in the weight of the fish. From the study it is very clear that higher levels of protein diet stimulated growth rate leading to the enhanced RNA synthesis in the growing tissues of *P. parrah*. This indicates that increased protein synthesis led to higher growth performance of fish under higher levels of protein treatment.

Table 6. Proximate composition* of *P. parrah* fed with protein diets

Parameters	Protein diets					
	30%		40%		50%	
	M	SD	M	SD	M	SD
Moisture	64.21±2.81		61.23±3.45		56.33±2.86	
Dry matter (%)	35.79±1.32		38.77±1.91		43.67±1.39	
Protein (%)	63.27±3.91		68.39±2.86		72.13±2.72	
Lipid (%)**	3.82±0.87		3.36±0.93		3.96±0.71	
Glycogen (%)	0.78±0.13		0.80±0.21		0.93±0.07	
Fiber (%)	4.42±0.88		4.42±0.92		4.41±1.02	
Ash (%)	6.12±0.99		6.21±1.02		6.61±1.12	
NFE (%)	21.59±1.21		16.82±1.25		11.96±1.20	

* Expressed on dry weight basis; ** Nitrogen Free Extract

a, b, c - Means with the same superscripts do not differ (Duncan's multiple range test)

In the present experiment parameters regarding the proximate composition of fish muscle showed an increasing trend from lower levels to higher levels of protein diets (Table 6). Fish fed with 50% protein diet showed highest protein deposit in the muscle. This may be due to the increased protein digestibility (Ramnarine, 1995) through enhanced protease activity of the digestive tract. Thus it could be suggested that the increased protein content observed in the muscle of *P. parrah* is due to the higher levels of protein in the diet. Higher protein deposition was also observed in common carp, rohu (Nanjundappa & Varghese, 1988, 1989), tilapia (Sindhu, 1992) and pearlspot (Sambhu & Jayaprakas, 1994). This increased deposition of protein showed that it was formed as a result of the increased protein synthesis. Similar high protein values were also observed in tilapia and pearlspot after different levels of protein diet administration (Steinhart, 1992).

The authors express their sincere thanks and gratitude to Prof. K. Sashikumar, Principal, Sree Narayana College, Kollam for the facilities provided. The authors are thankful to Dr. N.K. Balasubramanian, Reader in Biostatistics, Aquatic Biology and Fisheries, University of Kerala for his valuable help in data analysis.

References

Alexis, M. (1986) *Aquaculture*, **58**, 75

AOAC (1990) *Official Methods of Analysis*, 15th edn. Association of Official *Analytical Chemists, Washington DC, USA*

Bidhan, C.P. (1992) *J. Inland Fish Soc. India.* **24**, 50

Bier, N. (1962) *Methods in Enzymology*, Vol.1 Academic Press, New York

Bondi, A. & Spandorf, A. (1954) *Bt. J. Nutri.* **8**, 240

Bulow, F.D. (1970) *J. Fish. Res. Bd. Canada.* **27**, 2343

Ceriotti, G. (1955) *J. Biol. Chem.* **214**, 59

Dhange, K.P. (1968) *Biol Sci.* **2**, 610

Duncan, D.B. (1955) *Biometrics.* **11**, 1

El-Dahhar, A.A. & Lowell, R.T. (1996) *Aquaculture Res.* **26**, 451

Fish, G.R. (1960) *Hydrobiol.* **15**, 161

Giles, K.W. & Mayers, A. (1965) *Aus. J. Biol. Sci.* **93**, 36

Gunasekera, R.M., Shim,K.F. & Lam, T.J. (1996) *Aquaculture.* **146**, 245

Halver, T.A. (1973) *Fish Nutrition.* 205p. Academic Press, New York, USA

Hardy, R. (1980) *Fish feed formulation*, Paper presented in the FAO/UNDP Training course in Fish Feed Technology, Seattle, W.A., USA

Islam, S.Q. & Hossain, M.A. (1994) *Indian J. Fish.* **41**, 15

Kawai, S. & Ikeda, S. (1971) *Bull. Japn. Soc. Sci. Fish.* **37**, 333

King, J. (1965) *Practical Clinical Enzymology*, 363 p. Van Nostrand Company Ltd. New York, New Jersey, USA

Kiron, V. (1996) *Studies on some aspects of the culture of mossambique tilapia, Oreochromis mossambicus.* Ph.D. Thesis, University of Kerala, India

Kunitz, M. (1947 *J. Gen. Physiol.* **30**, 291

Mohanty, S.S. & Sumantara, K. (1996) *Environ. Ecol.* **11**, 625

Nanjundappa, T. & Varghese, T.J. (1988) *Proc. First Indian Fish Forum.* M.M. Joseph (Ed.) Mangalore, Karnataka, India

Nanjundappa, T. & Varghese, T.J. (1989) *Proc. Indian Acad. Sci. (Anim. Sci.)* **93**, 85

Nayak, N.P., Tripathi, S.D., Jain, K.K. & Srivastava, P.P. (1996) *Proc. Fourth Indian Fish. Forum.* M.M. Joseph (Ed.), Mangalore, Karnataka, India

Ramnarine, I.W. (1995) *J. Aqua. Trop.* **10**, 159

Sambhu, C. (1996) *The effect of hormones and growth promoters on the growth and body composition of pearlspot, Etroplus suratensis* (Bloch) and *white prawn, Penaeus indicus.* (M. Edwards). Ph. D. Thesis, University of Kerala, Thiruvananthapuram, India

Sambhu, C. & Jayaprakas, V. (2000) *Fish. Technol.* **37**, 109

Sherly (1997) *The effect of hormones and growth promoters on the grouth responses of fringed lipped carp, Labeo fimbriatus (Day).* PhD. Thesis, University of Kerala, Thiruvananthapuram, India

Sindhu, B.S. (1992) *Effect of dietary administration of protein and steroid hormones on the growth and reproduction of Oreochromis mossambicus,* M.Sc., Dissertation, University of Kerala, India

Snedecor, G.W. & Cochran, W.G. (1968) *Statistical methods.* Oxford and IBH Publishing Company, Calcutta, India

Steel, R.G.D. & Torrie, J.H. (1980) *Principles and Procedures of Statistics A-Biometrical Approach*, 481 p. McGraw Hill, New York

Stickney, R.R. (1972) *Principles of warm water aquaculture.* 373 p. Wiley-Inter Science Publication, John Willey & Sons Inc., India

Steinhart, D. (1992) *Aquaculture.* **92**, 68

Sumagaysay, N.S. & Borlongan, I.G. (1995) *Aquaculture.* **132**, 273