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EFFECT OF DIETARY PROTEIN ON THE GROWTH AND REPRODUCTIVE PERFORMANCE OF THE INDIGENOUS ORNAMENTAL FISH, *PUNTIUS VITTATUS* (DAY)

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Abstract:*Puntius vittatus*(Day) is a minor carp belonging to Cyprinidae family. It is a good aquarium fish and forage fish. The objective of the present study was to evaluate the impact of varying levels of dietary protein on the growth and reproductive performance of the indigenous ornamental fish, *P. vittatus* and to develop a protein balanced diet for the ornamental breeding system. Dietary sources of energy and nutrients are needed for growth, reproduction and health. Fish must have all of its necessary nutrients and supply of energy in optimum balance and quantity to perform optimally. Fishes were fed with fish meal based experimental diets containing 15, 25, 30, 35 and 45 percent protein. The results of the study show that dietary protein content necessarily influenced the growth and reproductive performance of fishes. *P. vittatus* fed with 35% protein diet attained greater length and weight than fishes fed with 15%, 25%, 30% and 45% protein diets during the period of experiment. The staging of gonads revealed that fishes fed 35% protein diet attained the ripe stage within seventy five days. The reproductive performance of *P. vittatus* in terms of G.S.I. and ova diameter measurement was the highest in fishes fed with 35% protein diet. However, there is a certain level beyond which further growth is not supported and the fishes fed with 45% protein diet showed reduced growth rate and reproductive development. The results of the study suggest 35% protein diet as optimum for growth and reproduction in the indigenous ornamental fish *P. vittatus*.

***Key words**: Puntius vittatus,* dietary protein, growth, reproductive performance

INTRODUCTION

Fish require dietary sources of energy and nutrients in optimum balance for growth, reproduction and health (De Silva and Anderson, 2009; Li, 2009; Lovell, 2009; Webster, 2009). The nutrient requirements vary between and within species. Fishes have developed a wide variety of feeding specializations to acquire essential nutrients and utilize varied food resources from the natural environment whereas in the case of culture systems a nutritionally balanced diet must be provided.Proteins are the major organic material in fish tissue constituting 65-75% of the total dry weight basis and are required for basic functions such as maintenance, growth and reproduction (ICAR, 2009). The concept of feeding fish is to nourish the animal to the desired level of growth and productivity. Somatic growth entails an increase in size of the body and reproductive growth entails an increase in size of gonads (De Silva and Anderson,2009). Adequate protein is essential for egg development, spawning, formation of follicles, ovarian tissues, growth and development of embryo(Shim *et al.*, 1989a). Reproductive performance and quality of eggs play a major role in developing ornamental fish culture and trade (Maya Devi, 1997). India is a country rich in indigenous species and is considered as the gold mine of ornamental fish resource. Organised export trade in ornamental fish depends on assured and adequate supply which is only possible by mass breeding.Detailed knowledge on feeding is one of the essential parameters in large scale production of ornamental fish (Gortemiller, 1993). *P. vittatus* is one of the potential indigenous ornamental fishes of India, it is peaceful, playful and easily bred aquarium fish and also forms a good forage fish relished by murrels, catfishes and anabantids (Innes, 1953). *P. vittatus* is in commercial trade and is placed under the vulnerable category in the IUCN Red List (Pethiyagoda, 1991). The present study was therefore undertaken to investigate the impact of different levels of dietary protein on growth and gonad development of *P. vittatus*.

MATERIALS AND METHODS

Puntius vittatus (Day) of Cyprinidae family was chosen as the experimental fish. Fish meal based experimental diets containing 15, 25, 30, 35 and 45 percent proteins were formulated following Hardy's (1980) method. The proportion of ingredients of the experimental diets is given in Table 1. and the proximate composition in Table 2.

Table 1. Proportion of ingredients of the experimental diets

	PERCENTAGE PROTEIN IN DIETS				
INGREDIENTS (g)	15	25	30	35	45
Fish meal	12.8	23.64	28.6	33.75	52.15
Groundnut oilcake	11.6	22.12	26.88	31.65	33.79
Rice bran	39.43	27.12	22.21	17.3	4.5
Tapioca powder	8	8	8	8	8
Cellulose	28.17	19.12	14.21	9.3	1.56
Vegetable oil (ml)	4	3	2.5	2	1
Vitamin mineral mix	1	1	1	1	1

Table 2. Proximate composition of the experimental diets

PARAMETER	PERCENTAGE PROTEIN IN DIETS				
	15	25	30	35	45
Protein (%)	15.65	25.51	30.34	35.16	45.08
Lipid (%)	7.1	6.8	6.88	6.95	7
Carbohydrate (%)	62.65	52.69	46.98	41.28	30.97
Ash (%)	12.14	13.15	13.98	14.82	15.12

The ingredients were finely ground and screened individually. The required quantity of powdered ingredients were weighed accurately, mixed well and kneaded to wet dough by adding sufficient water. This was then cooked for ten to twenty minutes under pressure, pelletized and oven dried at 60°C for 24 hours. The dried pellets were broken in to crumbles and stored at room temperature in air tight containers. The experiment was conducted in glass tanks of size 60cm×30cm×30cm. Three replicates were maintained for each treatment. The water level in the tank was maintained at 20±2 cm throughout the experiment.During the culture period the water temperature was measured to be 26°C; p^H 7 and dissolved oxygen 7.2mg/l. Fishes were collected from wild, brought to lab and stocked in stocking tanks for 10 days. The initial length and weight of the fishes were recorded and were randomly transferred to experimental tanks at a density of ten fishes per tank. The fishes were fed *ad libitum,* twice daily. Fishes were reared for a period of seventy five days. About twenty five percent of water was replenished once in two days. The fishes were sampled fortnightly for evaluating the growth and ovary development. Fifteen fishes were randomly collected from each treatment and their length and weight noted individually. Three specimens from each treatment were sacrificed during each sampling to record the maturity stages of the gonad. Gonads were classified in to different stages of maturity based on I.C.E.S. scale (Wood, 1930) as reproduced by Qayyam and Qasim (1964 a,b). G.S.I. of ovary was calculated after rearing for seventy five days, using the formula G.S.I.=(weight of the ovary / weight of the fish) x 100

Ova diameter of twenty-five vitellogenic oocytes were measured from the ovary of the fishes fed with thirty and thirty five per cent protein diets.

Table 3. Fortnightly mean weight (g) of fishes in the five treatments

	PROTEIN LEVELS				
DAYS	15%	25%	30%	35%	45%
0	0.80±0.05	0.80±0.05	0.80±0.05	0.80±0.05	0.80±0.05
15	0.84±0.25	0.94±0.12	1.05±0.14	1.23±0.26	0.87±0.18
30	0.87±0.25	1.09±0.21	1.25±0.19	1.35±0.24	1.01±0.18
45	0.91±0.18	1.14±0.14	1.39±0.09	1.58±0.21	1.04±0.09
60	1.00±0.37	1.29±0.17	1.39±0.23	1.63±0.27	1.08±0.25
75	1.11±0.25	1.35±0.24	1.39±0.18	1.65±0.36	1.17±0.09

Table 4. Fortnightly mean length (cm) of fishes in the five treatments

DAYS	PROTEIN LEVELS				
	15%	25%	30%	35%	45%
0	2.74±0.09	2.74±0.09	2.74±0.09	2.74±0.09	2.74±0.09
15	2.74±0.17	3.31±0.12	3.53±0.07	3.6±0.27	3.06±0.11
30	3±0.37	3.72±0.07	3.96±0.13	3.80±0.31	3.36±0.36
45	3.21±0.24	3.79±0.09	3.99±0.09	4.3±0.17	3.51±0.09
60	3.21±0.19	3.88±0.31	4.19±0.07	4.45±0.16	3.57±0.12
75	3.31±0.17	4.01±0.06	4.33±0.24	4.52±0.17	3.72±0.077

Table 5. Gonado- somatic index (G.S.I.)

PARAMETERS	15%	25%	30%	35%	45%
Body weight (g)	0.55	1.1	1.3	1.5	0.92
Weight of ovary(g)	0.01	0.03	0.09	0.108	0.017
G.S.I (%)	1.81	2.72	6.92	7.2	1.85

RESULTS

In the present study, the optimum protein requirement for gonad development and maturation of ovary for *P. vittatus* was found to be 35% protein diet.Results of the study also indicate that fishes fed with 35% protein diet had maximum net weight gain compared to 15%, 25%, 30% and 45% protein diets. Fortnightly mean weight (g) and length (cm) of fishes in the five treatments are given in Table 3 and 4. respectively.

The net weight gain of fishes was the maximum in 35% protein diet (0.84g) followed by 30% (0.58g), 25% (0.54g), 45% (0.36g) and 15% (0.31g). Four stages of ovary development such as immature, early maturing, late maturing and ripe could be recognised during the present experiment. After 75 days of experiment the fishes fed with 35%diet showed a ripe ovary, fishes of 30% and 25%protein diet produced maturing ovary and fishes fed with 15% and 45% protein diets remained in the immature stage. The highest G.S.I was obtained for fishes fed with 35% protein diet followed by 30%, 25% and 45% and 15%. The data is represented in Table 5. The ova diameter measurements of fishes fed with 30% and 35% protein diets were 0.37± 0.13 mm and 0.58±0.10 mm respectively.

DISCUSSION

Higher growth rate in fishes is often associated with a high dietary protein level. However, there is a certain dietary protein level beyond which further growth is not supported and may even decrease (Abbas *et al*., 2005; Debnath *et al*., 2007). Lovell (2009) observed that a dietary excess or deficiency of useful energy can reduce the growth rate. Several workers recommended 28-35% protein diets for warm water fishes (Ogino and Saito, 1970; Takeuchi *et al*., 1979; Lovell, 1980). The dietary protein level (35%) in the present study is higher than the values reported for *Labeo rohita*- 25% (Khan *et al*., 2005) and *Xiphophorus helleri*- 30% (Shirley *et al*., 2006) and lower than that reported for*Symphysodon sp.* 44.9- 50.1% (Chong *et al*., 2000) and *Tor putitora*- 45-50% (Islam and Tanaka, 2004). Varied dietary formulations, fish sizes and phylogenetic differences might be the cause of different protein requirements of the fishes (Luo *et al*., 2004; Tibbetts *et al*., 2000; Sa *et al*., 2006).Carnivorous fish usually consume diets containing 50% protein and have a very efficient system for excretion of nitrogenous waste produced by protein metabolism. Herbivorous fishes are expected to have lesser protein requirements (Bowen, 1987). The experimental fish, *P. vittatus* belongs to the herbivores guild and is expected to have a protein requirement less than 50%. The gonado- somatic index of the present study showed a peak in 35% protein diet. Similar results were reported by Rosy (1984) and Shim *et al*. (1989b).Dahlgren (1980) observed that low dietary protein resulted in decreased ovary weight and volume, increased protein in diet resulted in greater ovary size and weightin *Poecilia reticulata*. Similar results were reported by Maya Devi (1997) in *Trichogaster leeri*. These observations substantiate the present study in which 15% and 45% protein fed fish, the ovary remained immature, 25% and 30% protein fed fishes showed maturing gonad while 35% protein fed fishes, the gonad attained ripe stage with fully ripe oocytes. A portion of the digested protein is used as energy for maintenance and routine metabolism. The remaining energy is diverted for somatic or gonad tissue growth (James and Sampath, 2004). The reason for slow growth in fishes fed with 15%, 20% and 25% compared to 35% protein diets in the present study can be attributed to the meagre amount of protein left for conversion to flesh or gonad tissue as a major amount of consumed protein was used for maintenance. In the present study the food energy obtained from low protein levels were apparently not sufficient for fish to satisfy fully the requirements of both somatic growth and reproduction as evident from lesser weight gain and G.S.I in fishes fed with 15%, 25% and 30% protein diets.Diets can negatively influence the wellbeing of a fish by inducing nutrient deficiencies and imbalances (Cho *et al*., 1985). A significant fall in growth and G.S.I was observed at 45% protein diet, indicating that 35% protein diet satisfied the requirement and is considered optimum for achieving maximum somatic and reproductive growth. A similar trend has been observed in many other fish species irrespective of culture strategies (Kim *et al*., 2002; Kim and Lee, 2005; Wang *et al*., 2006). These results clearly indicate the growth depressing effect and decrease in the protein utilization beyond requirement level of dietary protein is a well documented phenomenon (Tibbetts *et al*., 2000; Catacutan *et al*., 2001; Yang *et al*., 2002; Deepak and Garg, 2003; Kalla *et al*., 2004; Sa *et al*., 2006). According to Phillips (1972)the fish body cannot utilise dietary protein once the optimum level has been reached. Excess protein in the diet could reduce the performance due to higher energy requirement for catabolism rather than protein deposition. Deamination of proteins produces amino groups which must be excreted. The elimination is done at the expense of energy resulting to the lesser utilization of energy for growth purposes (Siddiqui and Khan, 2009). The decline in growth performance at protein diet above 35% can be attributed to this fact. Thus, in the case of present study it can be said that the dietary protein beyond 35% could not be used for protein synthesis or tissue building in fish. The excess protein was being oxidised to produce energy to deaminate and excrete extra nitrogenous load from the body.

CONCLUSIONS

The study clearly shows that the quality of diets have a profound influence on growth, and better reproductive performance of indigenous freshwater ornamental fish *P. vittatus*. According to the results, a diet supplemented with 35% fish meal based protein is recommended for better growth, early gonad maturation and good reproductive performance of *P. vittatus*. The data generated during the present study will be useful in developing protein balanced diets for ornamental fish breeding systems. Since the actual dietary protein requirement in fish are also affected by factors such as protein quality, levels of lipid and digestibility further studies considering these factors are also recommended.

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