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Supplementation of Four Protein Percentage to the Basal Diet of Catfish (*Mystus cavasius*) Fingerlings: To Analyses the Optimum Level of Protein and Body Composition of Fish

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Abstract: Reducing feed cost and the pollution originating from feed have been principal matters in fish nutrition. Mixed feeding schedules have been proposed as one of the methods to reduce both of them and successfully tested in many fish species. The aim of the present study was to determine the mixed feeds containing 30, 35, 40 and 45% of protein content of four pelleted feeds was given to the four groups of cat fish (*Mystus cavasius*) and evaluating the optimum level of protein in the diet for this fish by studying the biochemical parameters of cat fish (*Mystus cavasius*) 60 days feeding schedules.

Key words: Chicken intestine, azolla algae, different percentage of pelleted diets, optimum growth, protein, total free sugar, lipid analysis, electrophorosis, cat fish (*Mystus cavasius*)

INTRODUCTION

Aquaculture is a powerful livelihood for a large section of economically under-privileged population in India. Fish culture is induced primary by the need for increased protein supply. Feed is considered as the most critical input for augmenting fish production. Protein content of feed appears to be the most important factor to increase production in fish breeding. India is important sources of proteins, vitamins and minerals, suitable for incorporation in fish diet. Several studies have been carried out on the development of formulated feed for the species under controlled culture system (Mukhopadhyay and Ray, 2001; Khan *et al*., 2004; Biswas *et al*., 2006). In almost all these studies, ingredients, such as fishmeal, soybean meal and groundnut oil cake were liberally used. All these materials are becoming prohibitively costly for their continued use for preparation of fish feeds; it is necessary to ascertain the quantitative requirement of dietary protein in order to reduce the cost of feeds. Fish species generally require higher level of dietary protein for optimum growth than poultry or cattle.

The major nutritional needs of a number of fishes of importance in aquaculture have been enlisted (NRC, 1983) and reviewed by several workers (Halver, 1979; Cowey, 1988).

De Silva (1988) suggested that in fish culture operations the highest recurring cost is the feed cost. A variety of feed ingredients of both plant and animal origin are used in the preparation of artificial diet in intensive aquaculture. Inclusion of animal matter has been found to improve the quality of fish diets. In this context use of non-conventional bio-waste chicken intestine for animal origin as a partial or complete dietary replacement for fishmeal protein.

Recently the utilization of aquatic plants having high food value are used to supplement fish food has taken new dimension for producing the much required animal protein at low cost (Lakshmanan *et al*., 1967). Azollae which grows in association with the blue green algae *Anabaena azollae* for plant origin; it perhaps the most promising from the point of view of ease of cultivation productivity and nutritive value (Lumpkin and Plucknett, 1982).

The quantity and quality of protein in the diet mainly influence fish growth. This has necessitated search for alternative sources available locally in the country. Carbohydrate content influences fish food costs due to its low prices and high abundance. Although, carnivorous fish have limited capacity to use dietary carbohydrate (Hemre *et al*., 1995; Deng *et al*., 2000), improved growth performance with high carbohydrate diets. In present study also the carbohydrate sources such as tapioca powder and rice bran were added to improve the energy content of the formulated feeds.

The aim of the present study was to compare the efficacy of this chicken intestine based formulated diets on growth, feed utilization and nutrient turnover from feed to fish flesh of cat fish *Mystus cavacius*. The study was also aimed at evaluating the optimum level of protein in

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the diet for this fish by studying the growth and biochemical parameters of *Mystus cavasius* fed with four different pelleted feeds for 60 days.

MATERIALS AND METHODS

Experimental diet preparation: Chicken intestine, tapioca powder, Azolla algae and rice bran were chosen to prepare the pellets. This selection includes both animal and plant proteins. The chicken intestine was selected because of rich protein and tapioca powder for carbohydrate richness. The ingredients were heated to about 70°C and then cooled to room temperature. Percentage composition of carbohydrate, protein and fat are tabulated (Table 1). Four formulated diets (containing 30, 35, 40 and 45% of crude protein approximately) were prepared by Square method (Santhanam *et al*., 1987) and analyzed for biochemical composition (Table 2). The four ingredients viz. chicken intestine, tapioca powder, Azolla algae and rice bran were dried in sun light, ground to fine powder. Measured volume of water was mixed to these feed mixtures and dough form was prepared for each then add with 1% of vitamin mixers, then put to a hand pelletizer fitted with a 3 mm diameter and sieved a pellets (spaghetti type) of all four formulated feeds (DI, DII, DIII and DIV) were sun dried and broken into small pellets, stored in airtight containers in refrigerator for use during feeding trial.

Protein content of muscle and liver was estimated by the method of Gornal *et al.* (1949) and lipid was estimated by the method of Barner and Blackstock (1973) and a total free sugar was estimated by the method.

Experimental design: The 50 individuals young ones of catfish *Mystus cavasius* were (6.6±2.5 cm) were procured from local pond and brought to the laboratory, acclimatized in large tanks containing river water. Fish were fed *ad libitum* with rice bran and groundnut oil cake (1:1 ratio) in the form of small dough, twice a day. A major portion of water was changed daily in order to avoid any accumulation of excretory products and unused feed which might cause further stress to the fish. Since, physico-chemical features of water have significant influence on the biodegradability and toxicity of pollutants, hydro-biological parameters such as temperature (26.0±4.0°C), pH (7.1±0.1), salinity

Table 1: Percentage composition of ingredients

				Diet (g/100 g)			
Ingredients	Carbohydrates (%)	Crude protein (%)	Crude fat (%)	I	II	III	IV
Chicken intestine	2.3	53.83	5.8	17.6	28.3	39.0	49.60
Azolla	6.2	36.53	2.2	17.6	28.3	39.0	49.60
Rice bran	37.0	25.00	17.9	32.4	21.7	11.0	0.38
Tapioca powder	60.2	18.46	1.0	32.4	21.7	11.0	0.38
*Vitamin mixture	-	-	-	1.0	1.0	1.0	1.00

*Vitamins: Vitamin A IP (as Acetate): 10000 IU, calciferol IP (Vitamin D3): 1000 IU, Thiamine mononitrate IP: 10.0 mg, Ribo flavine IP: 10.0 mg, Pyridoxine Hydro chloride IP: 3.0 mg, Cynocobalamin IP: 15.0 mcg, Nicotinamide IP: 100.0 mg, Calcium Panto thenate IP: 16.3 mg, Ascorbic Acid IP: 150.0 mg, Tocopheryl Acetate IP: 25.0 mg, Biotin USP: 0.25 mg, Minerals: Calcium Phosphate: 129.00, Magnesium Oxide light IP: 60.00 mg, Dried ferrous Sulphate IP: 32.04 mg, Manganese Sulphate IP: 2.03mg, Total phosphorous in the preparation: 25.80 mg; Trace elements: Copper Sulphate IP: 3.3 mg, Zinc Sulphate IP: 2.20 mg, Sodium Molybdate :0. 25 mg, Sodium Borate IP :0.88 mg

Table 2: Biochemical composition of 4 experimental diets

Diets	Ingredients	Crude protein (%)	Carbohydrates (%)	Fat (%)
I	Chicken intestine	9.47	0.40	1.02
	Azolla	6.43	1.08	0.38
	Rice bran	8.10	11.99	5.80
	Tapioca powder	5.98	19.50	0.32
	Total	30.00	32.90	7.52
II	Chicken intestine	15.23	0.65	1.64
	Azolla	10.34	1.75	0.62
	Rice bran	5.43	8.03	3.88
	Tapioca powder	4.01	13.06	0.22
	Total	35.00	23.49	6.36
III	Chicken intestine	20.99	0.90	2.26
	Azolla	14.25	2.42	0.86
	Rice bran	2.75	4.07	1.97
	Tapioca powder	2.03	6.62	0.11
	Total	40.00	14.01	5.20
IV	Chicken intestine	26.71	1.14	2.88
	Azolla	18.12	3.08	1.09
	Rice bran	0.10	0.14	0.07
	Tapioca powder	0.07	0.23	0.01
	Total	45.00	4.59	4.05

(0.6±0.02 ppt), total hardness (18.0±0.4 mg L^{-1}) were measured by following the guidelines of Anonymous (1993) and were maintained throughout the study period. After the acclimatized period over each individual weight and length was measured. The 40 pieces of healthy young once with an average weight of 4.4±1.6 g and an average (total) length of 6.6±2.5 cm were selected for the experiment and distributed in 4 glass aquaria (each having 50 L capacity) at a stocking density of 10 fishes per aquarium. Five fishes were used for analysis of initial whole body proximate composition. Aeration was continuously provided from air compressors through air stones daily and about 50% of water from each aquarium was replaced with clean stored tap water. The aquaria were maintained indoor under natural photoperiod conditions. The experimental fish were fed twice daily at 09.30 and 17.00 h at a fixed feeding rate of 2% of the total biomass for a period of 60 days. Fish were weighed and length measured at fortnightly intervals and feeding quantity was readjusted accordingly. Left over feed, if any was removed by siphoning 2 h post feeding. At the end of the experiment all the fishes from each aquaria were used for analysis of whole body composition.

Sample collection: At the end of study fishes were sacrificed (12 h after the last feeding) under cold conditions; blood sample was collected from *Mystus cavasius* by making cut at the end of caudal region of the fish in eppendorf tubes pre-rinsed with anticoagulant solution. They samples where diluted with PBS in 1:2 ratio. The diluted blood samples were centrifuged at 10,000 rpm for 5 min to remove the haemocytes and other tissue debris.

Blood samples were than stored at -40°C until use. Muscle/liver sample was collected by dissected out from same fish as quickly as possible and washed with double distilled water again washed with PBS buffer after wards homogenized glass rod homogenize with using PBS buffer the aqueous solution were centrifuged at 15,000 rpm for 5 min at 4°C and collected supernatant.

The muscle sample was than stored at -40°C until use. SDS, PAGE Slab gel was carried out by the method of Laemmli (1970).

Marker protein: Standard marker protein [Phosphorylase b (mw = 97,000), Bovine albumin (mw = 66,000), oval bumin (mw = 45,000) and Carbonic anhydrase (mw 29,000)] from Bio rad, U.S.A was used as standard marker to identify the molecular weight of the unknown protein. The molecular weight of different protein in the blood, muscles of all the group of fish were obtained by plotting the R_f value on the standard graph drawn on semi log paper.

RESULTS AND DISCUSSION

The importance of protein level in relation to the energy level of the diets for *Mystus cavasius* is evident in the present study as was found earlier for several other fish species (Garling and Wilson, 1976). For maximum growth of fish, an optimum protein content is necessary. The efficient utilization of protein, however depends on the availability of other dietary nutrients like carbohydrate and fat in approximate quantities. Protein requirement of rainbow trout has been reported to be 38% based on purified diets having relatively high-protein digestibility (Anonymous, 1993), practical trout feeds are formulated to include 42 and 48% protein (Hardy, 2002).

The present study revealed that the maximum growth in terms of live weight gain as well as the optimum protein content of *Mystus cavasius* was attained at 40% of dietary protein level (DIII). The optimum protein requirement of Indian major carps ranges between 30 and 45% (Mohanty *et al*., 1990). Fingerlings of *Labeo rohita* observed by Bairagi *et al.* (2002) for a reference diet containing 40% protein fish meal and diets containing different levels of duckweed leaf meal. In addition to ambient water quality condition, quality of the ingredients play crucial role in voluntary intake of a formulated diet. Growth rate was directly proportional to the dietary protein intake up to the required protein level but declined with further increment in dietary protein. It seems that up to the optimum level of dietary protein incorporation, the energy contributed through non-protein sources meets the normal maintenance; demand of fish and the available protein energy is spared for growth purpose. But the depressed growth beyond the optimum protein level indicates the insufficiency of energy from non-protein source.

The excess of protein feed is utilized to supply energy for growth purpose. The extra-energy expenditure towards the digestion of protein could also be another important growth depressing factor. Plant protein feedstuffs are often limited to fish (Cho and Bureau, 2001; Francis *et al*., 2001), so minimum amount of protein content of Azolla algae (32.36%) was used in this study. Present study also revealed that the experimental diet given to *Mystus cavasius* has improved the lipid level of muscles, liver and blood significantly (Table 3). Inclusion of animal matter has been already found to improve the diet for fish (Singh *et al*., 1980).

In the present investigation, the fish mean length, mean weight, % length gain and % weight gain of different groups of fish at the end of 60 days after feeding them were varying in % of protein concentration shown in Table 4. From the Table 4, length and weight increase

Table 3: Biochemical analysis of sample

	Muscle (mg g^{-1} wet weight)			Liver (mg g^{-1} wet weight)			Blood (mg/100 mL)	
Diets	Protein	TFS*	Lipid	Protein	TFS*	Lipid	TFS*	Lipid
I	20.79±3.25	12.82±2.94	10.41±3.14	11.72±2.99	9.85±2.60	10.83±2.14	215.38±60.67	298.50±130.81
II	27.24±7.75	22.55±5.55	26.03±6.05	32.83±7.71	21.56±3.31	36.72±8.30	430.25±103.85	447.75±157.33
III	36.21±6.45	24.64±6.01	30.17±8.71	47.31±8.65	25.82±3.60	50.31±4.98	515.38±89.19	713.09±157.32
IV	29.41±2.37	17.89±8.02	15.38±6.51	46.00±6.55	22.40±4.81	42.06±6.08	476.92±134.71	630.16±130.81

*TFS: Total Free Sugar

Table 4: Weight and length gain of catfish

Diets	Mean length gain (mm)	Length gain (%)	Mean weight gain (g)	Length gain (%)
I	5.90±0.73	9.16±2.02	0.53±0.12	11.73±2.62
II	8.00±1.80	10.90±2.90	1.03±0.11	18.91±2.83
III	9.50±2.00	12.33±2.61	1.58±0.15	22.23±3.43
IV	9.00±1.60	12.10±2.18	1.53±0.16	20.15±1.95

steadily with the increase of protein concentration up to 40% of protein diet, the optimum mean length gain was 9.50±2.0 mm and weight gain was 1.58±0.15 g was observed pellets fed with Diet III (40% of protein diet). These values have been decreased in spite of the increase in protein content of the diet DIV (45%). The same trend has been reported by earlier researchers like Sivakumar (1990) in *C. carpio*; Rajkumar (1999) in *O. mossamibicus*. Khan and Jafri (1990) found highest percent increase in weight when the catfish fed with 40% protein diet.

The use of tapioca in this experimental feed is not only the source of carbohydrate and protein but also it is used as a binder. Tapioca flour used as the binder consisted 14.9% protein, 33.7% carbohydrate, 0.9% fat, 8.89% ash and 11.6 J mg^{-1} of energy (Haniffa *et al.*, 1990). Protein content and essential amino acids are richer in animals than plants and hence chicken intestine was used, as a main composition of dietary protein, there is no cost.

The major advantage of this formulated diet is the incorporation of protein content in the muscles of fish to a greater level to form protein rich food. In this study, maximum level of muscle protein of *Mystus cavasius* was observed as 36.21±6.45 mg g^{-1} wet weight, liver protein was observed 47.31±8.65 mg g^{-1} wet weights at 40% protein diet confirming the optimum level of protein for this species. According to Haniffa *et al.* (1990) crude protein of whole fish carcass increased up to 60% from 56.7% dietary protein.

The sugar level of muscles increased up to DIII (40% protein diet) have 24.64±6.01 mg g^{-1} wet weight, this value was decreased DIV (45% protein diet) i.e., 17.89±8.02. The same trend was observed blood and liver sugar level, appropriately at the increasing level of protein in the diet. The maximum level of muscle lipid was observed 30.17±8.71 mg g^{-1} wet weights, liver lipid was observed 50.31±4.98 mg g^{-1} wet weights and blood lipid was 713.09±157.32 mg/100 mL of DIII i.e., 40% of protein diet. This value was decreased DIV (45% protein diet) (Table 4).

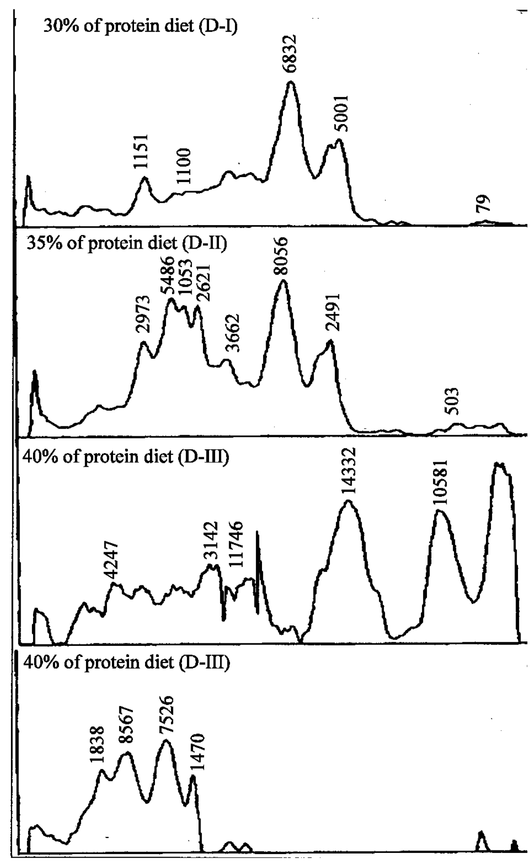

Fig. 1: Effect of four different diets (30, 35, 40 and 45% protein) on the serum protein profile (10% SDS-PAGE) of the *Mystus cavasius*

The electrophoretic separation and densitometric scanning of protein fractions of serum and muscle of the fish fed with 40% of diet contained many protein resolutions. This shows that high molecular weight proteins are added up in blood and muscle of *Mystus cavasius* when fed with optimum amount of dietary

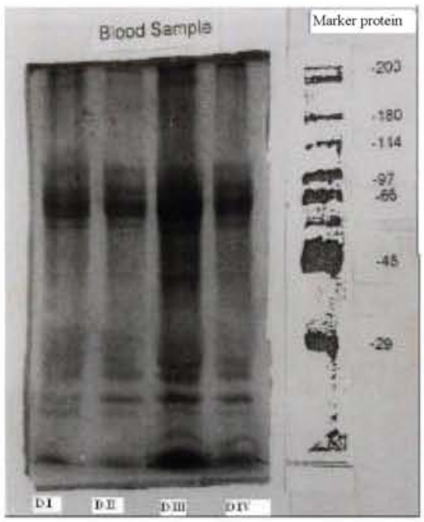

Fig. 2: Densitometric patterns of serum protein of four groups of *Mystus cavasius* fed with four different diets

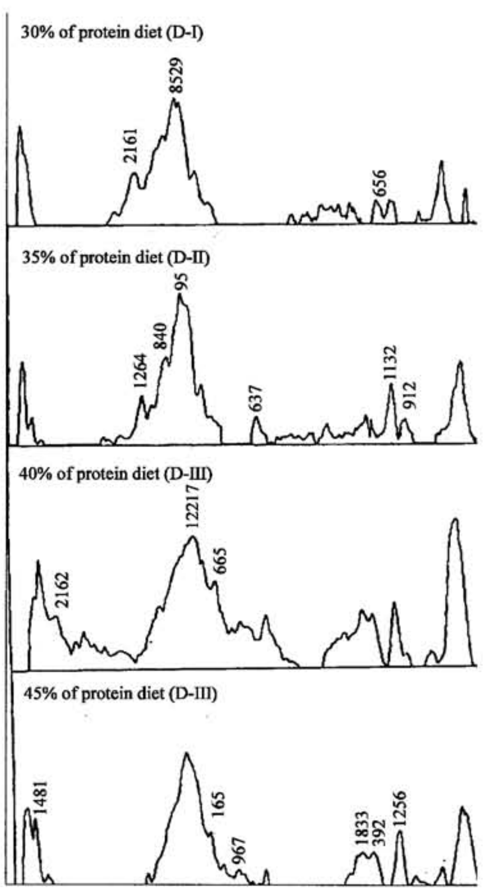

Fig. 3: Effect of four different diets (30%, 35, 40 and 45% protein) on the muscle protein profile (10% SDS-PAGE) of the *Mystus cavasius*

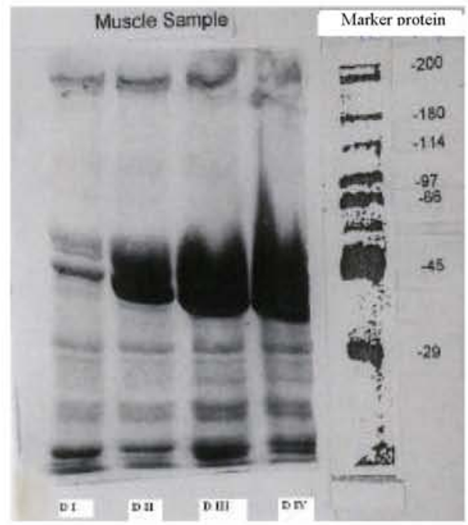

Fig. 4: Densitometric patterns of muscle protein of four groups of *Mystus cavasius* fed with four different diets

protein (40%). In the serum of the fish fed with 40% dietary protein showed the high molecular weight protein (100 kDa) and many fractions weighing, 78 and 66 kDa etc. These fractions are either absent or not clear in other groups (Fig. I and 2) the same trend has been reflected in the muscle of the fish fed with 40% dietary protein. This particular group revealed protein resolutions having the following molecular weights 198, 60, 27, 25, 16, 12 and 11 kDa (Fig. 3 and 4).

Further, the protein fractions in the middle phase of the gel are prominent in-group MIII and not clear in the group MII and MIV i.e., the group fed with 45% protein. The electrophoretic studies revealed that high molecular weight fractions are added up in blood and muscle of *Mystus cavasius* when fed with optimum amount of dietary protein (40%). This clearly suggests that both qualitative and quantitative increase in the protein content of tissue in this group. This study also revealed that there is qualitative improvement in the protein content of fishes, especially blood and muscle. As the dietary protein level increased the quality and quantity of protein also increased till the optimum level of 40% and then decreased in spite of the increase in the diet.

CONCLUSION

In this study, the experimental diet is superior over the conventional diet in increasing the protein content qualitatively and quantitatively and ultimately the growth of *Mystus cavasius*. In addition, the cost of food preparation was cheaper when compared with other commercial and traditional diets.

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