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Effect of Dietary Supplementation of Methionine on Growth, Survival and Immune Response of Indian Major Carp, *Labeo rohita*

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A B S T R A C T

The study was conducted in Cement cisterns of 25 m^3 size (5×5×1 m) with soil base to evaluate the effect of methionine, which is the indispensable amino acid on growth, survival and immune response of Indian major carp, *Labeo rohita*. The uniform size groups (109.07±2.6758 g) of rohu juveniles were used for the study. The study was carried out in five replicate groups for a period of 60 days. Fishes were stocked at the rate of 10 per tank. Two test diets namely T_1 and T_2 with 30% protein level were formulated. Diet T_1 had 0.08% and T_2 had 0.48% methionine, and diet without methionine supplementation served as a control (T_0). Significant differences were evident between treatment groups ($P<0.05$) in growth parameters, SGR, FCR. Among the tested doses 0.48% methionine showed better results than 0.08% methionine inclusion in the fish diet. Results of this study indicate that the best overall growth, survival and feed utilization of rohu fingerlings and juveniles were obtained with 0.48% methionine supplementation.

Introduction

Fish is a vital component of the human food supply and man's most important source of high-quality animal protein. Global fish production stood at 158 million tonnes in 2012 and fish consumption accounts for 17% of the global population's intake of animal protein which provides essential nutrients, vitamins and Omega-3 fatty acids, according to the Food and Agriculture Organization (FAO) State of World Fisheries and Aquaculture report released in 2014. While the global consumption of fish was 19 kg per person per year in 2012, for India it was 8 kg. The fisheries sector contributed 0.8% to India's gross domestic product (GDP) and 4.8% of the agriculture GDP in 2012-13. The global commercial production for human use of fish and other aquatic organisms occurs in two ways: they are either captured wild by commercial fishing or they are cultivated and harvested using aquacultural and farming techniques.

Among the Indian major carps, *Labeo rohita*, popularly known as "rohu", is one of the most widely cultured and preferred species in the country and commands higher price in the market. It is the natural inhabitant of freshwater sections of the rivers and thrives well in all fresh waters below an altitude of approximately 549 m. Rohu is a bottom feeder and prefers to feed on plant matter including decaying vegetation and attains maturity towards the end of the second year in ponds. The spawning season of rohu generally coincides with the southwest monsoon. Spawning takes place in flooded rivers. The fecundity of rohu varies from 226,000 to 2,794,000, depending upon the length and weight of the fish and weight of the ovary. The spawn of this fish is collected from rivers during monsoon and reared in tanks and lakes (Talwar and Jhingran, 1991). The economic success of fish culture depends on the cost of feeds, particularly the cost of protein materials incorporated into the feed. Prepared diets not only provide the essential nutrients that are required for normal physiological functioning but also may serve as a medium by which fish receive certain compounds that can alter endocrine activity, immunity and other physiological responses (Gatlin, 2002; Li *et al.*, 2005). Thus, in recent years there have been increased research efforts in developing dietary supplementation strategies, in which various growth promoting compounds like amino acids have been evaluated. Hence, the present study was undertaken to examine the optimum methionine requirements for juvenile rohu.

Protein is a major constituent of animal body. A liberal and continuous supply of protein is needed throughout life. The primary aim of fish culture is to efficiently transform dietary protein into tissue protein. Natural diets of fish are rich in protein. Generally, fish require a high percentage of protein in the diet than birds and mammals. This may be because, fishes utilize carbohydrates less efficiently. Therefore, some dietary protein may be metabolized for energy. The amount of dietary protein required by fishes is directly influenced by the indispensable amino acid pattern in the diet. The amount of protein needed to produce maximum growth has been investigated with purified test diets (H S Murthy, 1995, 1998; NRC, 1983).

In formulated feeds, protein is the most expensive component. Protein is a complex organic molecule consisting of more than twenty amino acids. When ingested protein is digested, amino acids are released into the body pool. They are absorbed for tissue protein synthesis. Methionine is one of the essential amino acids needed for good growth and health, but cannot be produced in the body, and so must be provided through the diet. It is a sulphur containing amino acid for growth of young fish (Wilson, 1989) and terrestrial animals (Visek, 1984). Quantitative dietary methionine requirements for several fish species have been shown to depend on dietary cystine concentrations. Cystine can substitute for a part of methionine requirement, since conversion of methionine to cystine is a common path way of intermediary metabolism in many terrestrial animals (Maynord and Loosli, 1979) and fish (NRC, 1973). The required amount of methionine has been investigated in common carp (Nose, 1979), chinook salmon (Halver *et al.*,1959),channel catfish (Harding *et al.*, 1977), rainbow trout (Rumsey *et al.*,1983), sea bass (Thebault *et al.*, 1985), channel catfish (Robinson *et al.*, 1978), blue tilapia (Liou, 1989), red drum (Moon and Gatlin, 1991), *Catla* (Ravi and Devaraj, 1991), milkfish (Borlongan and Coloso, 1993), rohu (Murthy, 2002).

Materials and Methods

Fish used in this study were Rohu (*Labeo rohita*) juveniles obtained from a commercial hatchery. These juveniles were conditioned for about ten days before the experiment, during which time they were fed a diet containing 30% protein. The experiment was conducted in cement cisterns of 25 m^3 size (5×5×1 m) with soil base at the College of Fisheries, Mangalore. The cisterns were thoroughly flushed with water and allowed to dry. They were then filled with freshwater drawn from an open well to a depth of 0.7±0.5 m prior to stocking and this level was maintained throughout the experimental period. Each cistern was stocked randomly with 10 fish weighing an average of 109.07±2.68 g and length 21.97 ±0.26 cm. Each diet was fed to five replicate groups of fish, at a rate of 2% of the body weight of the fish for 60 days. A minimum of 50% of the population was collected from each tank at every two weeks interval to record growth in terms of weight and length. Water samples from each cistern were analyzed every week for dissolved oxygen, free carbon dioxide, total alkalinity, ammonia and pH following standard methods (APHA, 1998). Water temperature ranged 28.5-31.5°C, alkalinity 42.27-49.05 ppm., dissolved oxygen 7.70-7.94 mg/l, pH 7.52-8.18 and total ammonia 0.19-0.23 μg N/l. The recorded parameters were within the range suitable for carp growth (Jhingran, 1991).

The ingredients used in the formulation of different experimental diets were soyabean meal, rice bran, corn flour, cottonseed meal, rapeseed meal, fish meal, sardine oil, vitamin mineral mixture, vit. C, methionine (MetAMINO). All the ingredients were procured from the local market except MetAMINO which was supplied by M/S from Evonik Degussa India Pvt, Mumbai and were analyzed for proximate composition prior to formulation of the test diets employing standard methods (AOAC, 2005).

Moisture content was estimated by heating samples at 105 ^{0}C for 30 min and then cooling and weighing to a constant weight. Crude protein was analyzed using Kjeltec system (Tecater 1002 Distilling Unit), fat content by Soxtech system (Tecater 1043 Extraction Unit), fibre content by using Fibretech System (Tecater 1017 Hot Extractor). The ash content was determined by first drying the sample and then heating it in a muffle furnace at 550 ± 10 ^{0}C for 6 h. Carbohydrate content was calculated as nitrogen free extract (NFE) by the difference method (Hastings, 1976).

Three experimental diets namely T_0, T_1 and T_2 with 30% protein content were formulated using the square method (Hardy, 1980). Diet T_1 had 0.08% methionine and T_2 had 0.48% methionine and without methionine supplementation served as control (T_0). The total daily ration of feed was divided into two equal meals and fed twice daily at the rate of 2% body weight during the experimental period.

The required quantities of ingredients were weighed accurately, mixed and hand kneaded to required consistency with just sufficient quantity of water (1:0.8) to get smooth dough. The dough so obtained was cooked under steam in a pressure cooker at 105^0 C for 20 to 30 minutes. The cooked dough was then cooled to room temperature rapidly by spreading in an enamel tray and required quantity of amino acid, sardine oil and vitamin-mineral premix were added, mixed and blended. The dough was extruded through a pelletizer having 3 mm diameter. Extruded pellets were dried in a hot air oven at 60^o C till the moisture content was reduced to less than 10%. The proximate compositions of the diets were determined according to standard methods (AOAC, 1995) and the amino acid compositions of the ingredients and diets were analyzed employing an amino acid analyzer (LKB model 415 Alfa plus). Diets were packed separately in high density polythene bags, labelled and stored in a dry place at room temperature for further use. Ingredients and proximate composition of the experimental diets are presented in Table 1.

The fishes were sampled once in a fortnight to assess the growth. The stocked fish were collected during each sampling and measured individually for growth parameters. After the experimental period of 60 days all the survived fishes were collected and their weight and survival data were recorded. The other growth parameters such as Absolute growth rate, Specific growth rate (SGR) and Feed conversion ratio (FCR) were calculated by using the following formulae.

Absolute growth rate (g) = Final mean weight – Initial mean weight

Relative growth rate (%) = [(Final mean weight – Initial mean weight) / Initial mean weight] x100

Specific growth rate (%) = [(ln final weight – ln initial weight) / Rearing period (day)] x100

Feed conversion ratio = Total dry feed offered (g) / Total wet weight gain (g)

The physico-chemical parameters of water such as temperature, carbon dioxide, dissolved oxygen, ammonia and pH in all the experimental tanks were estimated. Standard methods (APHA, 1998) were employed for the analysis of the water quality parameters.

Super oxide anion production assay (NBT assay) was analysed immediately after the completion of experiment. The test was performed as described by Anderson and Siwicki (1993) in flat bottomed microtitre plates. The activated phagocytes (neutrophils and macrophages) were characterized by their ability to adhere to glass or plastic and produce oxygen free radicals. NBT in its reaction with oxygen free radicals was reduced to blue formazan, the extent of which was determined by Enzyme Linked Sorbent Assay (ELISA) reader at 620 nm.

The experimental results were tabulated and analyzed statistically by using one-way analysis of variance (ANOVA) and Duncan's multiple range tests was used for mean separation (Duncan, 1955).

Results and Discussion

The result showed that dietary methionine levels had a significant effect on the final body weight, FCR, SGR and Super oxide anion production (Table 2 and 3). WG, SGR and Super oxide anion production (NBT) increased with the increasing dietary methionine level. Weight gained was significantly higher ($P < 0.05$) in methionine incorporated diet i.e. (314.00±8.12) in T_0 followed by T_1 (372.00±11.58) and T_2 (412.00±23.10). SGR also showed significantly increasing ($P < 0.05$) trend when compared to control diet, (1.69±0.04) in T_0 followed by T_1 (2.10±0.09) and T_2 (2.25±0.06). Super oxide anion production NBT significantly increased ($P < 0.05$) in methionine incorporated diet than that of control diet from (0.54±0.01) in T_0 followed by T_1 (1.12±0.15) and T_2 (1.67±0.06). The highest values of WG, SGR and Super oxide anion production (NBT) occurred at the 0.48% followed by 0.08% and control dietary methionine level ($P < 0.05$).

FCR decreased with increasing of dietary methionine level from (1.20±0.05) in T_0 followed by T_1 (1.03±0.03) and T_2 (1.01±0.03). There was no significant effect on length of juvenile rohu fed with methionine incorporated diet.

It is a common practice to supplement aquafeeds with methionine. In this study methionine was added to improve the rohu's growth performance. The WG, SGR, FCR and Super oxide anion production of the rohu fed the un-supplemented experimental diet was lower than those of the rohu fed the methionine diets. Supplementation of methionine and/or lysine in diets has shown to improve the weight gain of channel catfish raised in ponds or aquaria (Reigh, 1999) as well as sea bream (*Pagrus major*) (Takagi *et al.,* 2001).

Table.1 Ingredients and chemical composition of experimental diets

Diet ingredients (g/1000 g feed)	T_0 (Control)	T_1 (0.08% methionine)	T_2 (0.48% methionine)
Soybean meal	380.00	380.00	380.00
Rice bran	330.00	329.20	325.20
Corn flour	130.00	130.00	130.00
Cotton seed meal	50.00	50.00	50.00
Rape seed meal	50.00	50.00	50.00
Fish meal	30.00	30.00	30.00
Sardine oil	10.00	10.00	10.00
Vitamins and mineral mix[1] (Agrimin forte)	10.00	10.00	10.00
Vitamin-C	10.00	10.00	10.00
Methionine	-	0.80	4.80
Proximate composition (%)			
Dry matter (%)	91.02±0.04	91.38±0.04	91.33±0.01
Total moisture (%)	8.89±0.04	8.34±0.04	8.13±0.01
Total nitrogen	31.96±0.08	32.07±0.26	31.17±0.06
Crude protein	31.95±0.00	32.32±0.05	32.82±0.08
Crude fat	7.39±0.00	8.79±0.06	9.28±0.01
Crude fibre	7.09±0.03	7.01±0.04	7.19±0.14
Ash	12.63±0.02	11.19±0.10	10.87±0.02

[1]Vitamins and mineral mix (mg kg^{-1} feed) (Vitamin A 700000 IU; Vitamin D_3 70000 IU; Vitamin E 250mg; Nicotinamide 1000mg; Cobalt 150mg; Copper 1200mg; Iodine 325mg; Iron 1500mg; Magnesium 6000mg; Potassium 100mg; Sodium 5.9mg; Manganese 1500mg; Sulphur 0.72%; Zinc 9600mg; Calcium 25.5%; Phosphorus 12.75%)

Table.2 Weight gain, specific growth rate (SGR) and survival of rohu juvenile fed graded levels of methionine and control diet

Diet	Mean initial weight (g)	Mean final weight (g)	Mean weight gain (g)	Mean initial length (cm)	Mean final length (cm)
T_0	114.42±4.25	314.00±8.12^a	199.58±5.13^a	22.41±0.21	29.08±0.46^a
T_1	106.20±6.83	372.00±11.58^b	265.80±9.16^b	22.00±0.35	30.81±0.23^b
T_2	106.60±3.74	412.00±23.10^b	305.40±20.53^b	21.51±0.73	30.62±0.32^b

The means with different superscript in each column indicate a significant difference ($P < 0.05$). Each value is a Mean ± SE (n = 5 replicates).

Table.3 Weight gain, specific growth rate (SGR) and survival of rohu juvenile fed graded levels of methionine and control diet

Diet	FCR (%)	SGR (%)	Survival (%)	NBT-test
T_0	1.20±0.05^a	1.69±0.04^a	100	0.54±0.01^a
T_1	1.03±0.03^b	2.10±0.09^b	100	1.12±0.15^b
T_2	1.01±0.03^b	2.25±0.06^b	100	1.67±0.06^c

The means with different superscript in each column indicate a significant difference ($P < 0.05$). Each value is a Mean ± SE (n = 5 replicates)

Young ones of channel catfish required methionine at 2.34% of dietary protein in the absence of cystine for proper growth, (Harding *et al.,* 1977). Incorporation of methionine, lysine and cystine in diet enhanced FBW, SGR, FCR in Sea bass (*Dentrarshus laborax*) larvae (Salama *et al.,* 2013).

The FCR of the cobia fed the methionine diet was significantly lower than that of the cobia fed the coated methionine diets (Chi *et al.,* 2014).

Mc Cartney (1967), however, found that brook trout supplemented with 2% methionine showed improved growth and higher body protein when a high protein, high calorie diet was fed, but lower growth and body protein when fed a low protein and lower calorie diet. Similar results were obtained by Blaza *et al.,* (1982) in growing dogs where digestibility of energy improved significantly when methionine supplementation was increased in the diet.

Superoxide anion production is considered to be one of the most important microbicidal components and is an important index of immune function (Secombes, 1990).

Ali (2006) observed that, deficiency of methionine and lysine causes of all the blood parameters examined, the red blood cell count and mean cell volume showed significant decrease than the control.

Other haematological values were lower than that of the normal values. The RBCs and WBCs counts showed lower than the the control, also, Chaiyapoom *et al.,* (2006) noticed that, hematocrit value, Hb % were lower than the control with deficiency of methionine and/or lysine or with each other. In human, an amino acid mixture containing methionine was shown to increase plasma concentration of cholecystokinin and bile salt and lipase output (Colombel *et al.*, 1988).

The present study established that *Labeo rohita* fed with diet containing supplemented methionine showed a significant difference in growth and immune response. The growth, survival and immune response in *Labeo rohita* was higher in fish fed with methionine incorporated diets than in the control diet fed fish.

Among the two levels, 0.48% methionine showed better performance than 0.08% methionine.

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