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Effect of Methionine and Selenium on Growth and Nutrient Performance of Rohu (*Labeo rohita*)

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Abstract

The demand for fish is increasing due to its better nutritional profile. *Labeo rohita*, Hamilton (Rohu) is one of the major cultivable carps in India and it is important to enhance the production potential of this species. The effect of an essential amino acid (methionine, 0.5%) and mineral supplement (selenium, 0.5mg/kg) was studied to evaluate its efficacy on synergistic effect on growth performance of fish. Growth parameters of rohu were studied for 90 days in 4 separate groups – C (control with normal feed), T_1 (feed with methionine), T_2 (feed with selenium) and T_3 (feed with both methionine and selenium) with 3 replicates. Fish fry (average length of 25±2.46 mm; weight of 2±0.62 g) were stocked @ 20 per tank. Three test diets (T_1, T_2 and T_3) were formulated with 30% protein level. Significant differences were found among treatment groups (P<0.05) in growth parameters, proximate composition and liver enzyme activity. Among all groups, T_3 exhibited the best result with a food conversion ratio of 1.24±0.03, specific growth rate (%) of 2.95±0.019 and protein (%) of 17.18±0.02 followed by T_1 (FCR- 1.31±0.009; SGR- 2.75±0.014; Protein- 16.75±0.02) T_2 (FCR- 1.62±0.004; SGR- 2.46±0.024; Protein- 14.96±0.04) and C (FCR- 1.89±0.001; SGR- 2.07±0.021; Protein- 12.37±0.04). Results of this experiment indicate that the best overall growth and feed utilization of rohu fingerlings were obtained for T_3 as both methionine and selenium supplementation worked conjointly.

Keywords: FCR, SGR, protein, ALT, AST

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Introduction

The increase in fish growth depends upon the supply of quality feed containing an appropriate proportion of different nutrients. Among the Indigenous major carps, Rohu (*Labeo rohita*) is one of the most preferred species in India. It alone contributes to more than half of total fish production as it is suitable for both mono and polyculture (Hasan et al., 2013). Different essential amino acids help to reduce feed input costs as they are used in very small quantities without hampering fish growth (Murthy, 2002). Methionine has a central role as a building block in protein synthesis (Priyadarshini et al., 2018). Selenium (Se) is an important micronutrient that has been used in aquafeeds for the normal growth, welfare and health of aquatic animals. The application of Se in aquafeeds could improve the growth performance of aquatic animals (Khosravi-Katuli et al., 2017).

Several experiments have been carried out till date to observe the individual effects of methionine and selenium on fish regarding their growth, survival, immunity status etc (Priyadarshini et al., 2018; Khosravi-Katuli et al., 2017). However, the joint effect of these selenium and methionine may give better results. Hence, this experiment was carried out to understand the comparative effects of methionine-selenium combination on the growth and feed utility which can further help to formulate optimal feed for profitable aquaculture.

Materials and Methods

All the ingredients for feed formulation were procured from the local market. Three test diets (T_1, T_2 and T_3) were formulated (Table 1) with 30% crude protein for feeding the experimental fishes- C (control with normal feed), T_1 (feed with methionine), T_2 (feed with selenium) and T_3 (feed with both methionine and selenium). The proximate composition (%) of the formulated diet was carried out to know the moisture, crude protein, crude fat, nitrogen-free extract and ash content in the feed. Healthy fingerlings of rohu, (*Labeo rohita*) were obtained from nearby hatchery. The experiment was conducted in 12 FRP tanks (triplicate for each group-C, T_1, T_2 and T_3). Acclimatized fishes were then segregated and stocked in tanks @ 20 numbers per tank with almost uniform size (average length of 25±2.46 mm; weight of 2±0.62 g) so as to make the total weight of stocked fish per tank the same as per completely randomized design. Feeding was done twice a day (10 AM and 4 PM) at 4% body weight. The growth performance of *Labeo rohita* fingerlings was studied for 90 days. Fish sampling was done at every 15 days interval to record their growth in terms of weight and length. The growth indices such as growth and length increment, food conversion ratio, and specific growth rate were calculated. Proximate composition was also carried out to know the nutrient composition of the experimental fish after taking different test diets. Liver enzymes (ALT, AST) activity in blood were estimated using a colorimetric method of Reitmen & Frankel (1957). For analysis, two fish from each tank were sampled from the experimental tanks with minimum handling stress and transferred to the plastic buckets containing water of the same temperature and instantly anaesthetized with clove oil (40 μL/L) maintaining ethical procedure. The blood from the experimental fish was drawn using a 2 mL sterile syringe through a caudal vein puncture. The serum samples were collected by centrifugation at 2500 rpm for 15 min and transferred to Eppendorf tubes for measuring ALT and AST concentrations. The parameters were analyzed in the laboratory of the Department of Aquaculture of West Bengal University of Animal & Fishery Sciences. ANOVA and Duncan's Multiple Range post hoc analysis using the Tukey HSD test was carried out in CIBM-SPSS, version 22.0 considering the significance level at $p<0.05$.

Results and Discussion

The amount of protein in a formulated diet is the main nutritional element affecting fish growth. Numerous researches indicated the protein requirements of carps between 26% and 35% to have satisfactory growth (Ayyappan et al., 2006). In the present experiment, feed formulation was done with 30% crude protein content both in the control and experimental diet (Table 2).

Dietary methionine and selenium showed a significant effect on the growth parameters of the experimental fish. Final body weight, length and growth increment, FCR, and SGR showed significantly ($P<0.05$) better in diet with both methionine and selenium supplementation than the diet with

Table 1. Composition of experimental diet

Ingredients	Experimental Feeds (% Dry matter basis)			
	C	T_1	T_2	T_3
Rice Flour (g/100g)	10.0	10.0	10.0	10.0
Wheat Flour(g/100g)	22.5	22.5	22.5	22.5
Soyabean meal(g/100g)	30.0	29.30	30.0	29.30
Mustard oil cake(g/100g)	25.0	25.0	25.0	25.0
Fish Meal(g/100g)	4.0	4.0	4.0	4.0
Shrimp Meal(g/100g)	4.0	4.0	4.0	4.0
Vit-min. mix(mg/100g)	2.0	2.0	2.0	2.0
Fish oil(%)	2.0	2.06	2.0	2.06
Binder(g/100g)	0.5	0.64	0.5	0.64
Methionine(g/100g)	0	0.5	0	0.5
Selenium (mg/ 1kg)	0	0	0.5	0.5
Crude protein (%)	29.77	29.77	29.77	29.77
Ether extract (%)	5.67	5.67	5.67	5.67

Table 2. Proximate composition (%) of formulated diet

Contents	C	T_1	T_2	T_3
Moisture	6.46±0.020	7.31±0.006	7.38±0.004	7.10±0.009
Crude protein	30.60±0.004	30.61±0.021	30.65±0.008	30.64±0.006
Crude fat	11.43±0.005	11.44±0.006	11.48±0.003	11.42±0.004
Nitrogen free extract	39.19±0.002	39.19±0.003	39.19±0.002	39.19±0.007
Ash	12.02±0.032	12.21±0.004	12.34±0.007	12.24±0.005

*Data are presented as (Mean±SD)

Table 3. Growth parameters of experimental fish

Parameters	C	T_1	T_2	T_3
Mean initial length (cm)	$3.95±0.02^a$	$4.02±0.03^a$	$4.12±0.02^a$	$3.97±0.02^a$
Mean final length (cm)	$8.13±0.32^a$	$11.65±0.29^b$	$9.88±0.41^a$	$12.51±0.57^b$
Net increment in length (cm)	$4.18±0.12^a$	$7.63±0.19^b$	$5.76±0.09^{ab}$	$8.54±0.25^b$
Mean initial weight (g)	$1.77±0.002^a$	$1.98±0.007^a$	$2.01±0.004^a$	$1.73±0.011^a$
Mean final weight (g)	$8.73±0.012^a$	$13.1±0.11^b$	$11.7±0.023^b$	$16.01±0.093^b$
Net increment in weight (g)	$6.96±0.23^a$	$11.12±0.36^{ab}$	$9.69±0.31^a$	$14.28±0.38^b$
FCR	$1.89±0.001^b$	$1.31±0.009^a$	$1.62±0.004^b$	$1.24±0.003^a$
SGR (%)	$2.07±0.021^b$	$2.75±0.014^c$	$2.46±0.024^b$	$2.95±0.019^c$
Survival rate (%)	100	100	100	100

*Data are presented as (Mean±SD); Figures with the same superscript in a row are not significantly different ($p>0.05$)

Table 4. Proximate composition (%) of experimental fish

Contents	C	T_1	T_2	T_3
Moisture	81.66±0.23	77.95±0.14	79.44±0.25	77.64±0.34
Crude protein	12.37±0.04	16.75±0.02	14.96±0.04	17.18±0.02
Crude fat	3.77±0.01	3.60±0.05	3.72±0.03	3.54±0.07
Nitrogen free extract	0.6±0.0003	0.4±0.0001	0.4±0.0001	0.37±0.0002
Ash	1.6±0.002	1.3±0.001	1.48±0.002	1.27±0.002

*Data are presented as (Mean±SD)

Table 5. Liver enzyme activity of experimental fish

Enzymes	C	T_1	T_2	T_3
ALT (IU/L)	$22.05±0.27^a$	$21.11±0.19^a$	$24.2±0.86^{ab}$	$23.6±0.66^a$
AST (IU/L)	$19.1±0.11^a$	$17.25±0.26^a$	$22.15±0.19^b$	$21.09±0.24^{ab}$

*Data are presented as (Mean±SD); Figures with the same superscript in a row are not significantly different ($p>0.05$)

only methionine (Table 3). There was an increasing trend of weight and length in the treated groups than the control group. Weight gain (Fig.1) was highest in T_3 (14.28±0.38) followed by T_1 (11.12±0.36), T_2 (9.69±0.31) and C (6.96±0.23). FCR (Fig. 3) and SGR (Fig. 4) were also best in T_3 (Table 3). The length of the fingerlings (Fig. 2) also followed the same pattern with the highest increment in the T_3 group. There was no mortality of fish during the entire experimental period. Priyadarshini et al. (2018) also found the increasing SGR and growth of *Labeo rohita* with various levels of methionine supplemented diets. The incorporation of selenium (0.5 mg/kg diet) improved the growth performance and immunity of rohu (Ahmad et al., 2022). Selenium supplement also showed an improvement in growth and haematological parameters in *Labeo rohita* (Le et al., 2014).

Supplementation of the amino acid and the mineral creates variation in the nutrient content of the fish (Table 4). The highest protein content was found in T_3 (17.18±0.02) followed by T_1 (16.75±0.02), T_2 (14.96±0.04) and C (12.37±0.04). This variation was mainly due to the addition of methionine as it is the main building material of body protein as found by Murthy & Varghese (1998). There was no such variation of other nutrient composition in the experimental fish.

ALT and AST are the two most important biomarkers of healthy livers (Ballantyne, 2001). Both ALT and AST activity were highest in T_2 and lowest in T_1 (Table 5), which indicated that methionine is best for the healthy liver in fish. Methionine is a precursor to glutathione, a potent antioxidant that helps protect liver cells from damage caused by free radicals and toxins. It also prevents the accumulation of fat in the liver and maintains fat metabolism and the transport of fats out of the liver by choline synthesis. Overall, it plays a crucial role in supporting liver health by facilitating antioxidant activity, detoxification, fat metabolism, and protein synthesis (Li et al., 2020). A related result was reported in *Hypophthalmichthys molitrix* when fed with methionine-based selenium at an inclusion level of 0.9mg/kg diet (Mushtaq et al., 2022).

Conclusion

Results from the present experiment indicate that *L. rohita* fed with both methionine and selenium-enriched diet showed better growth rate and liver enzyme activity along with the highest protein content than other experimental groups with individual methionine and selenium-fortified diets. Though, there was no effect on the survival of the experimental fish, all the experimental groups showed better performance in each parameter than

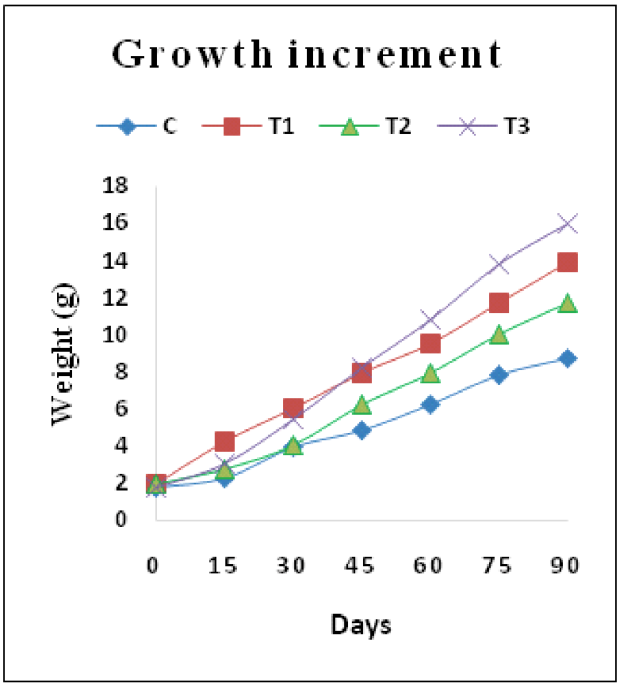

Fig. 1. Increment in growth

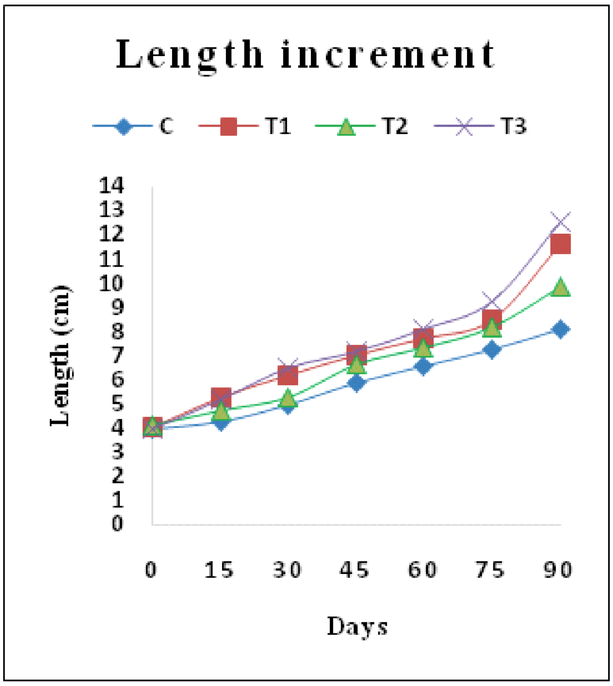

Fig. 2. Increment in length

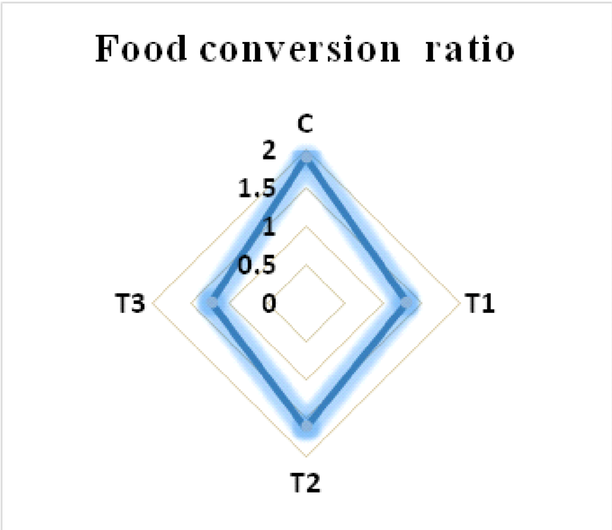

Fig. 3. Food conversion ratio of experimental fish

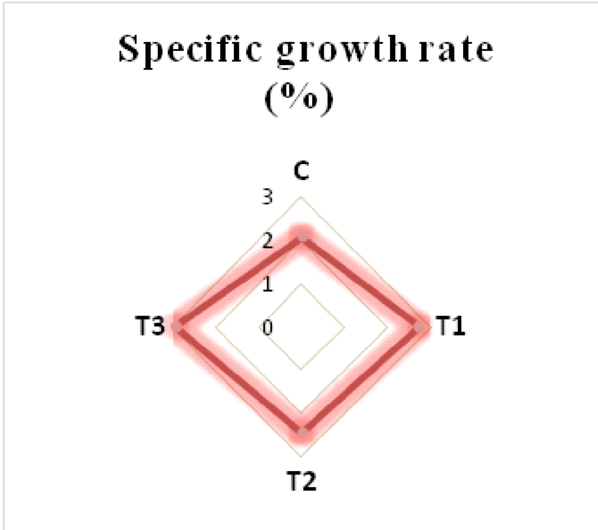

Fig. 4. Specific growth rate of experimental fish

the control group. However, further experiments should involve testing different species with varying doses of essential amino acids to develop an effective feed for sustainable growth. Additionally, research needs to be undertaken to understand the combined effect of these essential amino acids on enhancing fish disease resistance and immunity.

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