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Effect of Increasing Salinity on *Labeo rohita* Fed with Methionine and Selenium Fortified Feed

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Abstract Increasing global population is a main reason for elevated demand of fish as an animal protein source nowadays. The present experiment was carried out to increase fish production in a sustainable way and to make salt tolerant aquaculture candidate species by providing fortified feed without harming the natural environment. Methionine and selenium were used as feed supplementation for 90-day feeding trial in four 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). Then salinity tolerance test was carried out by adding sea salt in all groups with fresh water. Both Methionine and Selenium (T_3) showed the best result with the FCR of 1.24 ±0.003 followed by T_1 (1.31 ±0.009), T_2 (1.62 ±0.004) and C (1.89 ±0.001). Conjointly they also improved growth performance, proximate composition, haematological parameters and enzymatic activity in rohu. During the second phase, though experimental fishes were affected showing reduced growth performance and physiological activity, the hinderances were lesser in test groups than in the control group. In control, rohu showed less than 50% mortality in 6 ppt whereas T_3 showed the same at 10 ppt salinity. Thus, these two ingredients: methionine and selenium can be used to culture rohu in salt affected area with a greater possibility for improving production.

Keywords Rohu, Growth, Salinity, Haematology, Metabolic Enzyme

1. Introduction

Fish is an essential source of protein in everyday life. But the supply of fish is decreasing day by day due to the continuously overgrowing human population and fish stock depletion in natural water body. Harvest from capture fishery is following the same trend for over-exploitation of the natural stock. Increasing salinization in inland area also does not allow to grow more fish in freshwater region [1]. It mainly happens due to some anthropogenic activities like irrigation of salt affected agricultural land, industrial waste disposal and ion accumulation, the influx of seawater in freshwater region due to global warming, runout and leaching from the salt affected area etc. [2]. This salinity variation in inland water body creates stress on stenohalaine freshwater species [3]. It causes poor growth rate of the cultured species, changes in normal physiology and even mortality when salinity levels increase above their tolerance levels [4]. Though fish roughly use 10% of their energy in osmoregulation salinity affects the overall energy budget (20-50 %) in the fish [5]. Salinization is also reducing the area available for freshwater aquaculture. So, in this condition it is necessary to adopt suitable diversification in the culture process to increase optimum productivity of these areas [6]. Though culture of coastal water species in these salts affected inland area was practiced, different climate constraints and non-availability of a proper quantity of seed at the required time became the two most important hindrances in the non-coastal states especially in Northern India [7]. So, culture of freshwater species like carps and catfishes may be a master strategy to overcome the problem of increasing salinity in freshwater. To get optimum production, it is very necessary to study the salt tolerance levels of these species before introducing them in the culture area [2]. Different amino acids, vitamins and minerals help to control the energy budget in fish and supply the required strength to maintain the osmoregulatory functions. Methionine is the building block of the protein which increases the growth performance [8] and Selenium is an essential element which helps to reduce stress in unfavourable conditions [9]. In this context the present experiment was carried out to study the growth performance and salinity tolerance of *Labeo rohita* in increasing saline condition fed with Methionine and Selenium fortified diet.

2. Materials and Methods

Test diets (T_1, T_2 and T_3) were formulated with locally available feed ingredients with 30 % crude protein for feeding the experimental fishes viz C (control with normal feed), T_1 (feed with Methionine), T_2 (feed with Selenium) and T_3 (feed with both Methionine and Selenium). After acclimatization healthy fingerlings of rohu were stocked in triplicate tanks at the rate of 20 numbers per tank with almost uniform size (average length of 25±2.46 mm; the weight of 2±0.62 g) so as to make total weight of stocked fish per tank the same as per completely randomized design. Feed was given twice a day (10 AM and 4 PM) at the rate of 4% body weight. The feeding trial was done for 90 days in freshwater conditions. After that abrupt salinity challenge test was carried out by adding sea salt in all the experimental groups to know the required salinity to kill more than 50% fish in the experimental groups. For that reason, salinity of each group was increased by 2 ppt at an interval of 96 hours in each group. The survivability, salinity tolerance levels, growth performance (increment in length and weight, food conversion ratio, specific growth rate) along with different proximate parameters (proximate composition, haematological parameter and liver enzymatic activity) were estimated following standard protocols. The parameters were analyzed in the laboratory of Aquaculture department (WBUAFS). All the statistical analyses were done using statistical package tools for social sciences CIBM-SPSS, version 22.0 considering the probability level of $p<0.05$.

3. Results

3.1. Feed Composition

Numerous researches indicated the protein requirements of carps between 26 and 35 % to have satisfactory growth [10]. So, in the present experiment, feed formulation was done with approximately 30% crude protein content both in control and experimental diet (Table 1).

Table 1. Composition of experimental diet

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

3.2. Survivability in Saline Condition

In this study, the control feed fed fishes showed less salinity tolerance than the test diet fed fishes (Table 2). Generally, more than 50% mortality was found in 8 ppt salinity for control diet. There was 100% mortality in 10 ppt salinity. Whereas, in T_1, a diet fortified with Methionine, showed better performance than the control diet. There 100 % mortality was found in 12 ppt, but in 10 ppt the mortality was less than 50%. In T_2, nearly the same result was obtained but more than 50% mortality was found in 10 ppt salinity. But the T_3 experimental group showed the best result among all the test groups. Nearly 50% mortality was found in 12 ppt which is generally more than the other two experimental groups (Figure 1). This proved that Methionine and Selenium worked conjointly to mitigate the stress in rohu.

Table 2. Mortality of experimental fish in different salinity range

Mortality (%)	Salinity (ppt)							
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**
Control	Nil	Nil	Nil	10	80	100	-	-
T_1	Nil	Nil	Nil	Nil	10	40	100	-
T_2	Nil	Nil	Nil	Nil	20	90	100	-
T_3	Nil	Nil	Nil	Nil	Nil	20	70	100

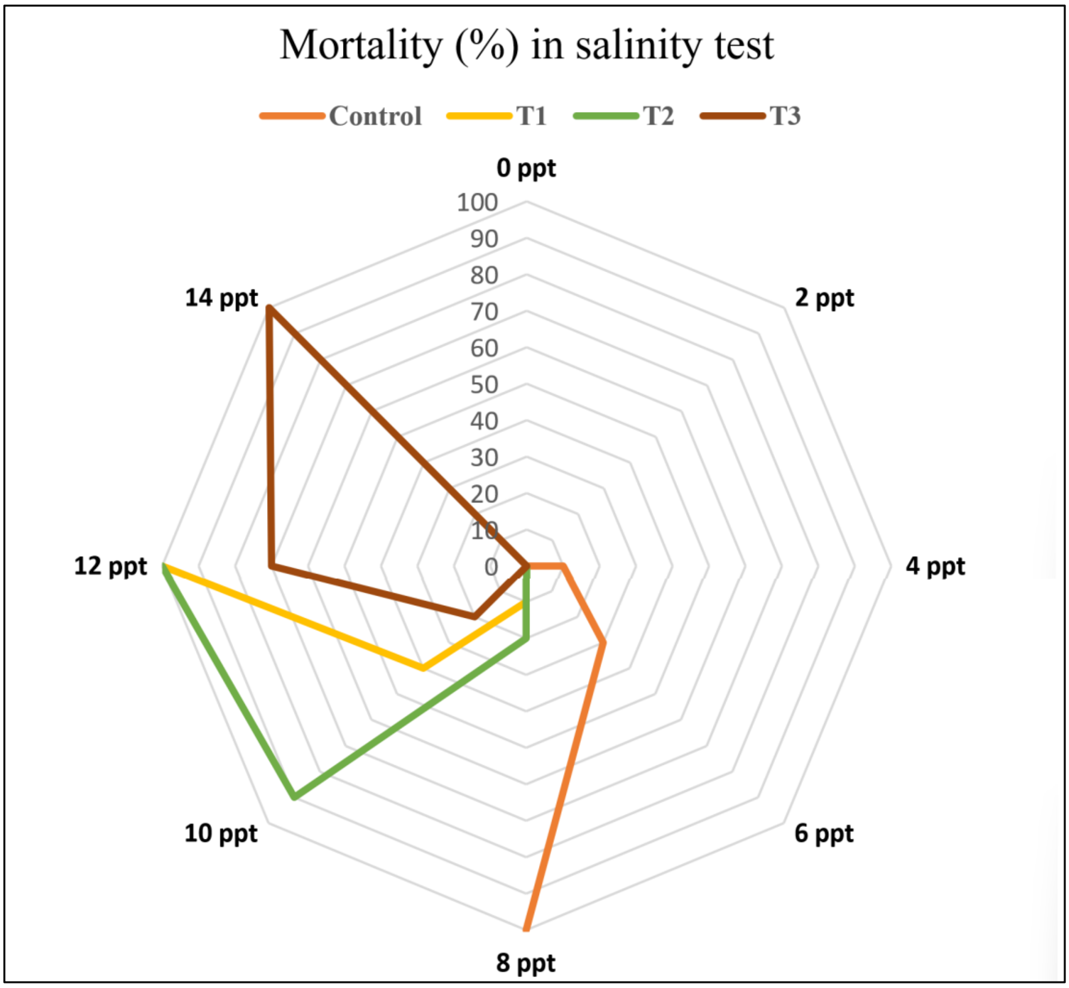

Figure 1. Mortality of experimental fish in salinity tolerance test

3.3. Growth Performance

The growth parameters, such as length increment, daily weight gain, Food Conversion Ratio (FCR) and Specific Growth Rate (SGR), were examined following both feeding trials and salinity challenge tests. In feeding trial, the treated groups exhibited an increasing trend in weight and length compared to the control group. Weight gain 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 and SGR also demonstrated superior performance in T_3. After the salinity challenge test, all experimental groups showed an increase in FCR and a decrease in SGR (Figure 2). However, the variation was less pronounced in T_3 (1.71 ± 0.04), followed by T_1 (1.97 ± 0.017), T_2 (2.39 ± 0.02), and C (2.78 ± 0.012). Overall, both methionine and selenium enhanced the salinity tolerance of rohu compared to their individual performances.

3.4. Proximate Composition

During feeding trial, 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). But in the second phase during salinity tolerance test, it was clear that protein content of fish decreased gradually with an increase in salinity content of water and the variation was significant ($p<0.05$) among all the trial groups. Although, lipid content was increased after salinity test the variations were very minute and the lowest was in T_3 followed by T_1, T_2 and the control group.

3.5. Haematological Parameters

The highest Red Blood Corpuscles (RBC) content was found in T_3 followed by T_1, T_2 and C. Both haemoglobin content and haematocrit value followed the same trend and platelets followed the opposite trend in freshwater conditions before salinity challenge test. Haemoglobin content, RBC content and haematocrit value significantly ($p<0.05$) declined with the increasing salinity concentration and were highest in the control group during salinity challenge test (Table 3). But there was no significance ($p>0.05$) found in the value of Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) in the treated groups during freshwater and saline water treatment.

3.6. Enzymatic Analysis

Both enzyme Alanine transaminase (ALT) and Aspartate aminotransferase (AST) activity were lowest in T_1 followed by T_3, T_2 and control in freshwater condition. It indicated that Methionine is best for a healthy liver. A related observation was found in grass carp when it was fed with Methionine-based Selenium dietary supplementation. But there was a significant difference ($p<0.05$) between ALT and AST activity in salinity test. There was an increasing trend in serum ALT value with increasing salinity in the experimental groups.

Table 3. Different growth parameters, proximate composition, blood parameters and liver enzyme activity observed in experimental fish

Groups	C		T_1		T_2		T_3	
Salinity (ppt)	**0**	**6**	**0**	**10**	**0**	**8**	**0**	**12**
Growth parameters								
Daily increment in length (cm)	0.045 ±0.002[a]	0.027 ±0.001[a]	0.084 ±0.002[a]	0.071 ±0.003[a]	0.064 ±0.002[a]	0.048 ±0.001[a]	0.09 ±0.002[a]	0.077 ±0.001[a]
Daily weight gain (g)	0.07 ±0.001[a]	0.02 ±0.001[a]	0.12 ±0.002[b]	0.09 ±0.002[a]	0.107 ±0.001[b]	0.84 ±0.003[b]	0.15 ±0.001[b]	0.11 ±0.002[a]
FCR	1.89 ±0.001[a]	2.78 ±0.012[b]	1.31 ±0.009[a]	1.97 ±0.017[a]	1.62 ±0.004[a]	2.39 ±0.02[b]	1.24 ±0.003[a]	1.71 ±0.04[a]
SGR (%)	2.07 ±0.021[a]	0.09 ±0.01[b]	2.75 ±0.014[a]	1.52 ±0.021[c]	2.46 ±0.024[a]	1.14 ±0.012[c]	2.95 ±0.019[a]	2.01 ±0.11[ac]
Proximate composition (% DM basis)								
Moisture	81.66 ±0.23[a]	81.21 ±0.45[a]	77.95 ±0.14[a]	77.02 ±0.23[a]	79.44 ±0.25[a]	78.85 ±0.21[a]	77.64 ±0.34[a]	77.19 ±0.24[a]
Crude protein	12.37 ±0.04[a]	11.45 ±0.14[a]	16.75 ±0.02[b]	16.32 ±0.09[b]	14.96 ±0.04[ab]	14.18 ±0.28[a]	17.18 ±0.02[b]	16.83 ±0.05[b]
Crude fat	3.77 ±0.01[a]	4.32 ±0.012[a]	3.60 ±0.05[a]	4.01 ±0.12[a]	3.72 ±0.03[a]	4.62 ±0.023[a]	3.54 ±0.07[a]	4.82 ±0.012 [ab]
Nitrogen free extract	0.6 ±0.003[a]	0.5 ±0.023[a]	0.4 ±0.002[a]	0.39 ±0.012[a]	0.4 ±0.0001[a]	0.35 ±0.02[a]	0.37 ±0.002[a]	0.3 ±0.02[a]
Ash	1.6 ±0.002[a]	2.05 ±0.012[a]	1.3 ±0.001[a]	3.69 ±0.03[a]	1.48 ±0.002[a]	2.62 ± 0.014[a]	1.27 ±0.002[a]	1.73 ±0.04[a]
Haematological parameters								
RBCs (10^6/µl)	0.76 ±0.042[a]	0.59 ±0.03[a]	0.93 ±0.05[a]	0.8 ±0.02[a]	0.81 ±0.02[a]	0.68 ±0.01[a]	1.02 ±0.04[b]	0.94 ±0.03[a]
HCT %	21.64 ±0.62[a]	20.49 ±0.14[a]	22.8 ±0.41[a]	22.07 ±0.23[a]	22.01 ±0.34[a]	21.03 ±0.24[a]	24.6 ±0.28[a]	23.99 ±0.36[a]
Platelets (10^5/µl)	7.19 ±0.14[b]	9.04 ±0.21[b]	6.02 ±0.24[a]	7.41 ±0.48[b]	6.84 ±0.41[a]	8.46 ±0.62[b]	5.56 ±0.51[a]	6.54 ±0.47[a]
Haemoglobin (g/dl)	6.6 ±0.11[a]	5.6 ± 0.25[a]	8.4 ±0.64[b]	8.01 ±0.45[b]	7.2 ± 0.74[ab]	6.5 ±0.54[a]	9.8 ±0.89[b]	9.57 ±0.92[b]
MCV (fl)	77.2 ±1.12[a]	74.6 ±1.01[a]	83.3 ±1.81[b]	81.2 ±1.62[ab]	79.14 ±1.27[a]	77.8 ±0.99[a]	87.2 ±1.41[b]	86.8 ±1.22[b]
MCH (pg)	50.58 ±0.89[a]	50.13 ±0.85[a]	51.86 ±0.92[a]	51.64 ±1.01[a]	51.01 ±0.72[a]	50.61 ±0.77[a]	52.02 ±0.45[a]	51.78 ±0.82[a]
MCHC (g/dl)	55.8 ±0.74[a]	61.11 ±0.81[b]	53.9 ±0.98[a]	57.6 ±0.46[a]	52.8 ±0.77[a]	57.23 ±0.51[a]	52.2 ±0.23[a]	54.83 ±0.41[a]
Liver enzyme								
ALT (IU/L)	24.82 ±0.27[a]	49.86 ±0.19[b]	22.86 ±0.19[a]	53.24 ±0.68[c]	24.2 ±0.86[a]	58.43 ±1.02[c]	23.12 ±0.66[a]	61.21 ±0.55[c]
AST(IU/L)	23.01 ±0.11[a]	56.21 ±0.92[c]	17.25 ±0.26[a]	58.73 ±0.84[c]	22.15 ±0.19[a]	61.22 ±0.87[c]	21.09 ±0.24[a]	74.02 ±0.68[c]

*Data are presented as (Mean ±SD)

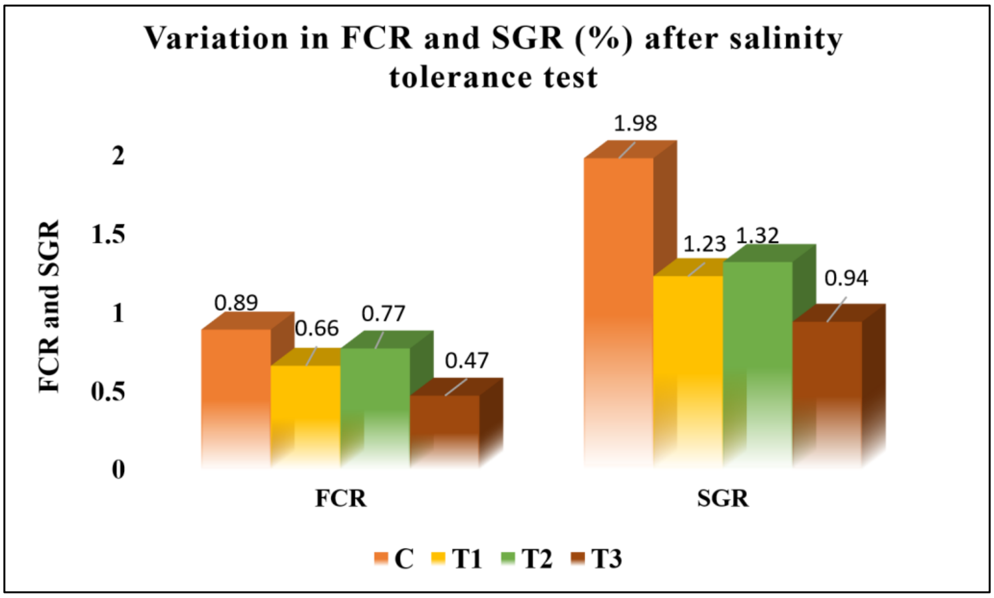

Figure 2. Variation of increasing FCR and decreasing SGR (%) between freshwater test and salinity tolerance test

4. Discussion

4.1. Survivability in Saline Condition

Freshwater stenohaline fish can normally survive up to 6 ppt salinity with very little mortality [3]. So rohu can be cultured well up to 6 ppt saline water. Methionine fortified diet affects the protein metabolism which is closely related to adaptation in saline environment which was found by Xu [11] in GIFT. Selenium and nano-Selenium help to reduce salinity stress in fish as they are good osmoprotectants [12,13]. Better survivability in a saline environment was obtained in the T_3 group and a similar result was found in *Hypophthalmichthys molitrix* by Mushtaq [14] with a Methionine-based Selenium diet in freshwater conditions.

4.2. Growth Performances

Two supplemented nutrients helped to enhance growth performance of rohu in the present experiment. Methionine supplementation improves the growth and metabolism of rohu as proved by Priyadarshini [8]. Ahmad [15] also found that Selenium incorporation in feed increased the growth performance of rohu. But after salinity tolerance test the result varied significantly. This result is similar to the findings of Rani and Gulia [16] in *Cirrhinus mrigala*. Xu [11] found the increase in protein metabolism and its effect on salinity adaption in GIFT after using Methionine supplementation. *Ictalurus punctatus* also showed higher SGR in freshwater than in saline water which is similar to the present findings [17].

4.3. Proximate Composition

As a building block of protein, Methionine helps to increase the essential protein component in carps. Jointly with Selenium, Methionine improved the growth performance of rohu as found in grass carp (*Ctenpharyngodon Idella*) with Methionine-based Selenium diet by Mushtaq [14]. Result of 2nd phase of experiment was similar to the findings of Barman [18], where protein content of milk fish (*Chanos chanos*) was lesser in 15 ppt than 0 ppt. In each experimental group, there was an increase in lipid content with increasing salinity concentration. But the moisture content followed the opposite trend with a decreasing rate with increasing salinity, as there is an inverse relationship between crude fat and moisture content of fish [19]. Increasing lipid content is an indicator of free energy usage which helped to mitigate the stress faced by rohu in saline condition [20].

4.4. Haematological Parameters

Haematological parameters are the main indicators of the physiological condition of fish in a stress condition. There is a decrease in RBC value when osmotic changes cause the leakage of ions from plasma [21]. But compared to freshwater conditions, the platelet counts were more in fish cultured in saline water. Platelets are able to produce different compounds affecting phagocytosis during stress. Increased platelet count is the indicator of stress response in saline environment [22]. This finding is similar to the results of Akinrotimi [23] where there was an increased level of platelets in *Tilapia guineensis* at higher salinity levels. But there was no significant difference in MCV, MCH, and MCHC in both freshwater and saline water experiments of Akinrotimi [23].

4.5. Enzymatic Analysis

Liver enzymes Alanine transaminase (ALT) and

Aspartate aminotransferase (AST) are the two most important indicators of liver health in fish. They are very important for protein metabolism. ALT and AST release into blood increasing its transaminase activity when there is an injury in liver and myocardial cell [24]. Serum AST is an indicator of several tissue damages [25]. It showed enhanced levels along with increasing salinity. The experimental observation was found similar with the result of Hoseini [26] with rainbow trout (*Oncorynchus mykiss*). So, according to this present experiment the increased levels of ALT and AST along with increasing salinity indicated that salinity can create hepato-cellular damage in fish.

5. Conclusions

From the present experiment, the findings can conclude that *Labeo rohita* fed with Methionine and Selenium fortified diet showed better growth performance, proximate composition, haematological parameters and enzymatic activity conjointly than individual effect. Though rohu can survive well up to 6 ppt salinity, these amino acids and minerals both individually and collectively can increase its salinity tolerance. They can survive well in 10 ppt salinity with less than 50% mortality. Though growth performance and other parameters were hampered due to stress created during increasing salinity, the effect of both Methionine and Selenium mitigated it in a better way. This finding opens a wide scope to culture rohu easily in brackish water areas without any hindrance. Field trials may be done with commercially important species of both freshwater and brackish water species to find the best combination for polyculture to improve production in a sustainable way.

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Conflict of Interest

All authors have no conflicts of interest.

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