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Effects of Exogenous Multi Enzyme on the Growth, Blood Parameters and Flesh Compositions of *Labeo Rohita* (H)

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Abstract: *As exogenous enzymes inclusion in aqua-feed is increasing in our country, a study was conducted to evaluate the effects of a exogenous multi enzyme on the growth, blood parameters and flesh compositions of Labeo rohita for 90 days feeding trail in twelve cages set in the pond of Department of Fisheries, University of Rajshahi, Bangladesh. The study was conducted under 4 treatments with 4 types of feeds prepared by using aquazyme plus (trade name) at the rate 0.0, 0.5, 1.0 and 1.5 g/Kg feed treated as T1 (Control), T2, T3 and T4, respectively. The study was carried out through measuring growth and feed utilization parameters (Weight gain, SGR, FCR and survival rate), blood parameters (WBC, RBC, Hb, PCV, MCV, MCH and MCHC) and flesh compositions (moisture, crude protein, lipid, ash and carbohydrate) of the fish according to the standard methods. During the study period, there was no significant difference in the water quality parameters among the treatments and were within the suitable range. Significantly higher weight gain and SGR were recorded in T4* (*461.37±2.23* g and *1.28±0.04 %)* and the *lower in T1 (321.62±13.1 g and 1.03±0.027 %). The better FCR was found in T4 followed by T3, T2 and T1. There was no significant difference in survival rate among the treatments. Exogenous enzyme inclusion in feed had significant effects on blood parameters of the fish. Moisture, ash and carbohydrate contents of the fish flesh were increased with the increasing dose of enzyme in the feed whereas crude protein and lipid contents were decreased. The present study concluded that the use of exogenous multi enzyme in feed increase the growth, change the blood parameters and flesh compositions of L. rohita.*

Key words: *Exogenous enzyme, feed, effects, growth, blood, flesh composition*

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I. Introduction

As fish is a chief source of high class protein, accelerating the development of fish culture technique is an important factor to meet up protein demand for increasing population of Bangladesh. But, the successful fish culture depends upon the use of nutritionally adequate and economically viable feeds. Increasing trend of fish culture in the country has expanded different feed industries where feed ingredients from animal sources are altered by plant sources to reduce the cost of feed. But, the plant based feeds have digestibility problem [1] due to high molecular weight non-starch polysaccharides within plant cell walls which increase digest viscosity, thereby reducing digestive enzyme access to other nutrients [2]. This can result in reduced feed efficiency and lower growth rate of fish. To minimize the digest viscosity of the feeds, different exogenous enzymes like phytase, proteases, lipases, galactosidases can be added to the feeds. Exogenous enzymes addition in feed enhance protein and carbohydrate digestibility that improve feed conversion and growth of fish [3][4]. Enzymes supplementation can help to eliminate the effects of anti-nutritional factors and improve the utilization of dietary energy [5].

Labeo rohita is a popular carp species cultured in Bangladesh due to its higher growth rate and they thrive well on artificial feed. Due to the higher consumer preference and market demand of large size fish, the farmers of the country use a verity of exogenous enzymes in feed to promote growth of *L. rohita.* Unfortunately, some of the farmers use these enzymes indiscriminately without knowing their necessity and proper dose. Sometimes representatives of different pharmaceuticals company influenced the farmers to use their products. The unregulated use of these enzymes could pose negative impacts on fish growth and flesh compositions of fish that remain unaddressed in Bangladesh.

However, a number of researches have been conducted on the effect of exogenous enzymes on the growth of fishes in different countries of the world [4][6][7][8][9] but, the researches on the effects of exogenous enzyme on the growth of carp fishes are so scare in Bangladesh. Therefore, the study was conducted to evaluate the effects of exogenous multi enzyme mixed feed on the growth, blood parameters and flesh composition of *L. rohita* in Rajshahi, Bangladesh.

II. Materials and Methods

2.1 Study location and period

The study was conducted in 12 cages that were set in the pond situated at the North side of the Department of Fisheries, University of Rajshahi, Bangladesh for a period of 90 days from September to November, 2018.

2.2 Preparation of experimental feeds

Four experimental feeds were prepared by adding AquaZyme Plus (Each 100 gm contains, â-Glucanse: 330000, Xylanase : 220000, Pectinase : 40000, Cellulase: 15000, Acid Protease: 3000, Neutral Protease: 3000, Mannase: 2500, Gluco Amylase: 2400 and Amylase: 50 IU) at the rate of 0.0, 0.5, 1.0 and 1.5 g/kg feed. The enzyme treated feeds were coded as Feed-1 (Control), Feed-2, Feed-3 and Feed-4, respectively. The proximate compositions of the experimental feeds are shown in Table-1. There was no significant difference in proximate compositions among the experimental feeds.

Table 1: The proximate composition of experimental feeds

Component (%)	Experimental Feeds			
	Feed-1 (Control)	**Feed-2**	**Feed-3**	**Feed-4**
Moisture	12.69±2.12	12.71±1.61	12.74±1.72	12.67±1.93
Crude protein	31.84±2.11	31.89±2.08	31.93±2.09	31.97±1.93
Lipid	6.44±1.67	6.35±1.64	6.37±1.53	6.33±1.49
Ash	14.43±1.98	14.41±1.93	14.35±1.87	14.36±1.93
Carbohydrate	34.60±2.10	34.64±2.13	34.61±2.01	34.67±2.07

2.3 Experimental design

The study was carried out under four treatments viz., Treatment-1 (T1), Treatment-2 (T2), Treatment-3 (T3) and Treatment-4 (T4) with three replicates in each treatment. T1 was assigned to the fish treated with Feed-1 (Control, feed without enzyme), T2 was assigned to the fish treated with Feed-2, T3 was assigned to the fish treated with Feed-3 and T4 was assigned to the fish treated with Feed-4.

2.4 Collection and raring of fish

One hundred ninety two juvenile of *L. rohita* were purchased from a local fish farm and transferred to the experimental pond in the scientific way. The fish were acclimatized to the experimental condition for one week before the start of the trail. Sixteen fish per cage were randomly released in T1, T2, T3 and T4, respectively. Fish were fed twice daily with 4% of body weight. During the feeding trail, fish was weighed fortnightly and the ration size was adjusted.

2.5 Monitoring of water quality parameters

During the feeding trail, water temperature, DO, pH, total alkalinity and NH_3-N were measured fortnightly with standard methods.

2.6 Analysis of growth and feed utilization

The initial and final weights of fish in each group were measured individually. Sampling of the fish was done fortnightly in order to record weight of fish. Weight gain, specific growth rate (SGR), feed conversion rate (FCR) and survival rate (SR) was calculated according to standard formulae [10] as follows:

1. Weight gain = final weight − initial weight
2. SGR = 100 (Ln (average terminal BW) − Ln (average initial BW)/test days)
3. FCR = weight gain/feed consumption
4. SR = number of fish at end of test/number of fish on first day of test × 100

2.7 Analysis of blood parameters

Blood samples of the fish were taken from the caudal vein in a haematocrit tube containing anticoagulant agent. Total count of white blood cells (WBC) and red blood cells (RBC), haemoglobin level (Hb), pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed. Total WBC and RBC were counted by an improved Neubaeurhaemocytometer [11]. Hb level and PCV were determined by cyanmethemoglobin method [12] and microhaematocrit method [13], respectively. The value of MCV was calculated according to Feldman *et al.* [14]. MCH and MCHC values were calculated according to Stoskopf [15].

2.8 Chemical analysis of feed and fish flesh

Moisture, crude protein, lipid, ash and carbohydrate content of the experimental feeds and fish flesh were analyzed using standard method [16].

2.9 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) to test for the level of significance through SPSS software. The significance difference among the mean values were determined by Duncan's multiple range test at level 5% ($P<0.05$).

III. Results

3.1 Water quality

The recorded values of water quality parameters viz., temperature, DO, pH, total alkalinity and NH_3-N did not show any significance variation among the treatments.

3.2 Growth and feed utilization

After 90 days of feeding trail, the variation of weight gain, SGR, FCR and survival rate summarized in Table-2. Significant differences were observed in the growth parameters and feed utilization of fish among the treatments. From the result it was observed that *L. rohita* fed feed with aquazyme plus resulted in increase body weight gain and SGR compared to the control fish. The fish fed with aquazyme plus at 1.5 g/kg (T4) had the highest body weight gain and SGR compared to other fish. Feed conversion rate improved significantly with increasing aquazyme plus levels and the best FCR value (lower) was recorded in T4. No significance difference was observed for survival rate of fish among the treatments.

Table 2: Growth performance and feed utilization of the fish under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
Initial weight (g)	210.33±4.40^{a}	211.33±3.25^{a}	211.25±4.88^{a}	212.52±3.67^{a}
Final weight (g)	531.95±11.14^{c}	559.92±18.01^{c}	606.98±11.53^{b}	673.89±19.67^{a}
Weight gain (g)	321.62±13.1^{c}	348.59±18.3^{c}	395.73±8.62^{b}	461.37±2.23^{a}
SGR (% bwd^{-1})	1.03±0.027^{c}	1.10±0.072^{bc}	1.17±0.006^{b}	1.28±0.04^{a}
FCR	2.69±0.015^{c}	2.59±0.20^{b}	2.57±0.021^{b}	2.20±0.015^{a}
Survival rate (%)	88.00±3.35^{a}	92.00±4.45^{a}	88.00±4.00^{a}	92.00±4.00^{a}

* T1 was assigned to the fish treated with Feed-1 (Control, feed without enzyme), T2 was assigned to the fish treated with Feed-2, T3 was assigned to the fish treated with Feed-3 and T4 was assigned to the fish treated with Feed-4. Values in the same row with different superscripts are significantly different ($P<0.05$).

3.3 Blood parameters

The current study showed that exogenous multi enzyme mixed feed exerted a certain influence on the blood parameters (Table-3). Difference was observed in total WBC, RBC, Hb, PCV, MCV, MCH and MCHC values among the treatments. Total WBC, RBC count and PCV values in the fish treated with enzyme mixed feed (T2, T3 and T4) were significantly higher than the control fish (T1) and the highest value was found in fish treated with 1.5g aquazyme plus feed (T4). Hb values were insignificantly increased in the treated fish than the control fish. MCV and MCH values in T2, T3 and T4 were significantly decreased than the control fish (T1). Moreover, MCHC values in T1 and T4 showed no significant difference but the values in T2 and T3 were significantly different from T1 and T4.

Table 3: Blood parameters of the fish under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
WBC ($\times 10^4$ mm^{-3})	7.22±0.27^{c}	8.51±0.11^{b}	8.73±0.15^{b}	10.29±0.5^{a}
RBC ($\times 10^6$ mm^{-3})	2.07 ±0.05^{b}	2.29±0.02^{a}	2.38±0.18^{a}	2.40±0.15^{a}
Hb (g/dl)	6.83±0.12^{a}	6.90±0.10^{a}	6.98±0.11^{a}	6.96±0.13^{a}
PCV (%)	24.39±0.35^{b}	26.24±0.98^{a}	34.11±1.93^{a}	34.70±1.25^{a}
MCV (fL)	84.65±3.98^{a}	65.66±0.37^{b}	68.94±0.51^{b}	72.21±5.1^{b}
MCH (pg)	26.57±1.53^{a}	11.24±0.98^{c}	10.47±2.35^{c}	19.01±0.50^{b}
MCHC (g/dl)	28.10±2.16^{a}	17.66±0.45^{b}	14.17±1.65^{c}	26.7±2.36^{a}

* T1 was assigned to the fish treated with Feed-1 (Control, feed without enzyme), T2 was assigned to the fish treated with Feed-2, T3 was assigned to the fish treated with Feed-3 and T4 was assigned to the fish treated with Feed-4. Values in the same row with different superscripts are significantly different ($P<0.05$).

3.4 Flesh composition

Changes in flesh compositions of *L. rohita* fed the feed with exogenous enzyme are shown in Table-4. Crude protein and lipid contents of fish flesh were significantly decreased in T2, T3 and T4 compared to T1 and the lowest value was recorded in T4. Contrary, moisture, ash and carbohydrate contents were significantly increased in T2, T3 and T4 when the fish fed feed with enzyme compared to the control fish (T1).

Table 4: Carcass compositions of fish under four treatments

Components (%)	Treatments			
	T1	T2	T3	T4
Moisture	7.78 ± 0.24^{c}	8.74 ± 0.08^{a}	8.90 ± 0.03^{a}	8.60 ± 0.08^{b}
Protein	74.96 ± 0.27^{a}	71.94 ± 0.19^{b}	71.44 ± 0.13^{c}	70.61 ± 0.65^{d}
Lipid	8.14 ± 0.15^{a}	7.86 ± 0.08^{b}	6.62 ± 0.03^{c}	6.54 ± 0.05^{c}
Ash	6.79 ± 0.24^{c}	7.66 ± 0.44^{b}	11.23 ± 0.15^{a}	11.59 ± 0.08^{a}
Carbohydrate	0.63 ± 0.06^{d}	1.23 ± 0.04^{c}	1.65 ± 0.62^{b}	2.64 ± 0.19^{a}

*T1 was assigned to the fish treated with Feed-1 (Control, feed without enzyme), T2 was assigned to the fish treated with Feed-2, T3 was assigned to the fish treated with Feed-3 and T4 was assigned to the fish treated with Feed-4. Values in the same row with different superscripts are significantly different ($P<0.05$).

IV. Discussion

4.1 Growth performance and feed utilization

Inclusion of exogenous enzymes in the feed appeared to have positive effects on the growth of fish [17]. In the present study, different doses of an exogenous multi enzyme, aquazyme plus (trade name) added to a commercial feed in order to investigate the possible effects on the growth and feed utilization of *L. rohita*. Lower doses of aquazyme plus in feed (0.5 g kg-1 feed) showed no significant effects on fish growth while comparatively higher doses of aquazyme plus (1.0-1.5 g kg-1 feed) increased the growth in significant manner compared to the control fish. According to current study, the optimum growth performance and feed utilization (higher weight gain, SGR and lower FCR) were obtained in the fish fed feed with higher level of enzyme (1.5 g/kg feed). The results are in the agreement with Bogut *et al.* [18] who found that exogenous enzyme significantly increased the growth of *Cyprinus carpio*. Likewise, inclusion of exogenous enzyme in Nile tilapia diets resulted in improved growth parameters [19]. Supporting evidence of the current study have also been found in the reports of different researchers who studied the positive effects of various multi-enzyme complex on the growth of *Oreochromis mossambicus* [20], Caspian salmon [4], *Huso huso* [21] and of *Labeo rohita* [22]. Although, most studies on other species indicate that exogenous enzymes improve weight gain and feed conversation rate [23][24][25], some conflicting reports are also present suggesting no or even adverse effects when these enzymes were added to animal feeds [26][27] which might be due to enzymes used in their study were partly different from the current research.

4.2 Blood parameters

Blood parameters are an important indicator of the physiological state of the internal organs. Blood parameters of fish are useful tool to assess the health status and as well providing information on nutrient status, digestive function and routine metabolic level of fish [17]. Changes in blood composition can be result of changes occurred in diet quality and nutrient compounds [28]. The current study indicates that inclusion of exogenous enzyme in feed affects blood parameters of *L. rohita*. Total count of WBCs was increased significantly in aquazyme plus treated fish compared to the control fish. Thus, the use of exogenous enzyme led to the higher WBC count indicating that multi-enzymes are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production [29]. Results of current study are in agreement with Zamini *et al.* [4], Shakoori *et al.* [17] and Haghbayan and Mehrgan [30]. Red blood cells, hematocrit and hemoglobin are responsible to carried oxygen in body. In this study RBC and PVC/Haematocrit values were increased significantly whereas Hb levels increase insignificantly in aquazyme plus treated fish compared to the control fish. However dietary exoenzyme in *L. rohita* diet is associated with an increase in RBC number, Hb level and hematocrit level in plasma. The results of the current study are more or less consistence with findings of Goda *et al.* [31] who cited that red blood cells counts, hematocrit and hemoglobin of Nile tilapia were significantly highest in all treatments receiving exogenous digestive enzymes supplemented-diets. Suggestion reason for this influence may be attributed to the increased ALP enzyme activity in liver, that cause improve immune system function as well as transport activity. Moreover, enzyme inclusion in the feed had considerable effects on MCH, MCHC and MCV values in the treated fish compared to the control fish.

4.3 Flesh compositions

Flesh compositions of *L. rohita* fed with aquazyme plus mixed feeds were affected significantly. The fish fed with feed containing 1.5 g/kg aquazyme plus showed the highest values of moisture, ash and carbohydrate content and the lowest values of lipid and protein content compared to the control fish. Generally, fat content shows inverse relation with moisture content [32]. Body fat of *L. rohita* decreased when the fish fed with aquazyme

plus mixed feeds and the fish had the lowest fat content (6.54±0.05%) when the fish fed with 1.5 g/kg aquazyme plus mixed feed, on the contrary it showed highest moisture content compared to the control fish. This finding is in accordance with the findings of Goda *et al.* [31] and Adeoye *et al.* [33] in Nile tilapia and Haghbayan and Mehrgan [30] in rainbow trout. A tendency of lower flesh protein content and higher ash content with increasing percentage of aquazyme plus in feed was seen in this study. Similar findings have been reported by Goda *et al.* [31] in Nile tilapia and Ghomi *et al.* [21] in *Huso huso*. Haghbayan and Mehrgan [30] also found decrease in flesh protein contents in rainbow trout upon feeding with exogenous enzyme. With the inclusion of aquazyme plus in feed, carbohydrate content in treated fish increased compared to the control fish. Though, this finding cannot be justified with any other relevant studies, but it might be due to the exogenous enzyme had influence in carbohydrate absorption and assimilation. It has been claim that higher enzyme levels could liberate excessive amounts of monosaccharides [34]. In contrast, low levels of enzymes may increase viscosity of the digesta by increasing the soluble non-starch polysaccharides, resulting in reduced digestibility and absorption [35].

V. Conclusion

The present study sated that the use of exogenous multi enzyme in feed increase the growth and feed conversion rate, change the blood parameters and flesh compositions of *L. rohita*. Moreover, studies including more doses of enzyme in the feed and extended trail period are required to draw more accurate conclusion on the effects of exogenous multi enzyme mixed feeds on the growth, haematology and flesh composition of, *L. rohita*.

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