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Inclusion of Vitamin-C in a Commercial Feed of *Labeo rohita* (H): Effects on Growth, Blood Parameters and Carcass Compositions

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Abstract: *A study was conducted to investigate the effects of vitamin-C enriched feed on the growth, blood parameters and carcass compositions of Labeo rohita (H) for a period of 3 months from September to November, 2018 in twelve cages set in the pond of Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. The study was conducted under 4 treatments with 4 types of feeds prepared by using vitamin-C at the rate 0, 200, 300 and 400 mg/Kg feed treated as T1 (Control), T2, T3 and T4, respectively. The study was carried out through measuring growth and feed conversion parameters (Weight gain, SGR, survival rate and FCR), blood parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC) and carcass compositions (Moisture, crude protein, lipid, ash and carbohydrate) of the fish according to the standard methods and formulae. During the study period, there were no significant differences in the water quality parameters among the treatments and were within the productive ranges. Significantly higher weight gain and SGR was recorded in T3and the lower in T1. There was no significant difference in the survival rate among the treatments. The better FCR was found in T3 followed by T4, T2 and T1. Vitamin-C enriched feed had significant positive effects on the blood parameters of the fish. Significantly higher crude protein in carcass was recorded in the fish treated with higher doses of vitamin-C whereas the higher lipid content was recorded in the fish treated with lower dose of vitamin-C. Overall study revealed that the inclusion of dietary vitamin-C in the commercial feed at 300 mg/kg feed had positive effects on the growth and FCR, blood parameters and the carcass nutrients of L. rohita.*

Key words: *Inclusion, vitamin-C, commercial feed, effects, growth, blood, carcass*

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I. Introduction

Fisheries has been playing an important role in addressing nutritional security of people in the developing countries. Fish being one of the major sources of animal protein and have helped greatly in improving nutrition in Bangladesh. Thus, accelerating the development of fish culture industry is essential to fulfill the protein demand for increasing population of the country. The success of fish culture depends largely on the quality feeds. Scaling up of fish culture expanded different feed industries in recent years of the country. Unfortunately, most of the feed industries failed to meet up the standards for nutrients requirement of fish, especially micronutrients requirement like vitamins and minerals.

Vitamins as important micronutrient are required for the maintenance of normal body functions of fish. Proper amount of vitamins are required for the normal metabolic processes within the enzymatic system [1]. Among the vitamins, vitamin-C is strong antioxidant and is considered as one of the essential micronutrient required for growth and immunity of fish [2]. Vitamin-C is necessary for optimum growth and body maintenance [3]. Vitamin-C has an extensive role in the enhancement of growth, collagen synthesis, iron metabolism, reproduction, stress physiology, wound healing and immune response in fish [4].

However, *Labeo rohita* is an important culture species in Bangladesh due to its higher nutritive value and high market demand. This carp species is omnivore in nature which can be cultured with relatively less effort. As vitamins are now used as fish feed additives to promote production and health condition of fish, the production, health condition and muscle quality of *L. rohita* can be improved by enrichment of feed with proper amount of vitamin-C.

Although, a numbers of research works have been done on the effects of dietary vitamin-C on the growth and survival rate of different fish species in different parts of the world [5][6][7][8] but, the researches on the effects of vitamin-C enriched commercial feed on the growth, health condition and carcass compositions of *L. rohita* are rare in Bangladesh. Therefore, the aim of the study is to investigate the effects of dietary vitamin-C for the growth, blood parameters and flesh composition of *L. rohita.*

II. Material and Methods

2.1. Experimental site and period

The study was conducted in 12 cages that were installed in the pond situated at the North side of the Department of Fisheries, University of Rajshahi for a period of 3 months from September to November, 2018.

2.2. Preparation of experimental feeds

Four experimental feeds were prepared by adding vitamin-C trade name VER C (Vision Agro Pharma, Bangladesh) in a commercial feed at the rate of 0, 200, 300 and 400 mg/kg feed. The feeds were coded as Feed-1, Feed-2, Feed-3 and Feed-4, respectively. The proximate compositions of the experimental feeds are shown in Table-1. There is no significant difference in the proximate compositions of the experimental feeds ($P<0.05$).

Table-1: Proximate compositions of the experimental feeds

Component (%)	Experimental Feeds			
	Feed-1	Feed-2	Feed-3	Feed-4
Moisture	12.70±2.45[a]	12.73±1.99[a]	12.71±2.48[a]	12.70±2.12[a]
Crude protein	31.81±2.24[a]	31.76±2.05[a]	31.72±2.87[a]	31.69±2.48[a]
Lipid	6.38±1.22[a]	6.32±1.08[a]	6.30±1.30[a]	6.31±1.23[a]
Ash	14.43±1.99[a]	14.45±2.17[a]	14.47±2.53[a]	14.48±2.13[a]
Carbohydrate	34.68±2.17[a]	34.74±2.13[a]	34.80±2.20[a]	34.82±2.33[a]

❖ *Values are mean of triplicate determination.*

2.3. Experimental design

The study was carried out under four treatments viz., T1, T2, T3 and T4 with three replicates in each treatment. T1 was assigned to the fish treated with Feed-1 (Control, feed without vitamin-C), T2 was assigned to the fish treated with Feed-2 (200 mg vitamin-C/kg feed), T3 was assigned to the fish treated with Feed-3 (300 mg vitamin-C/kg feed) and T4 was assigned to the fish treated with Feed-4 (400 mg vitamin-C/kg feed).

2.4. Collection and raring of experimental fish

One hundred ninety two juvenile of *L. rohita* were purchased from a local fish farm and transferred to the experimental pond by a van in a proper scientific way in the presence of aeration system. The fish were acclimatized to the experimental condition for one week before the start of the experiment. 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 experimental period, fish were weighed fortnightly and the ration size was adjusted.

2.5. Analysis of growth and feed conversion

The initial and final weights of fish in each group were measured individually. Weight gain, specific growth rate (SGR), feed conversion rate (FCR) and survival rate (SR) was calculated according to standard method [9] as follows:

- Weight gain = final weight − initial weight
- SGR = 100 {Ln (average terminal BW) − Ln (average initial BW)/test days}
- FCR = weight gain/feed consumption
- SR = (number of fish at end of test/number of fish on first day of test) × 100

2.6. 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 [10]. Hb level and PCV were determined by cyanmethemoglobin method [11] and microhaematocrit method [12], respectively. The value of MCV was calculated according to Feldman *et al.* [13]. MCH and MCHC values were calculated according to Stoskopf [14].

2.7. Chemical analysis of feed and fish carcass

Moisture, crude protein, lipid, ash and carbohydrate content of the experimental feeds and carcass were analyzed using standard method [15].

2.8. Monitoring of water quality parameters

Water temperature, DO, pH, total alkalinity and NH_3-N were measured fortnightly with standard methods. The variations in the water quality parameters under four treatments are presented in the Table-2. During the study period, there was no significant variation in the water quality parameters among the treatments ($P<0.05$).

Table-2: Variation in the water quality parameters under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
Temperature (°C)	29.65±0.75[a]	28.42±0.82[a]	29.44±0.61[a]	29.19±0.90[a]
DO (mg/l)	6.25±0.46[a]	6.41±0.57[a]	6.14±0.34[a]	6.10±0.35[a]
PH	7.45±0.12[a]	7.42±0.86[a]	7.37±0.051[a]	7.42±0.08[a]
Alkalinity (mg/l)	141.50±2.74[a]	146.83±4.07[a]	143.17±10.25[a]	142.67±5.54[a]
NH_3-N (mg/l)	0.032±0.004 [a]	0.037±0.01 [a]	0.039±0.002 [a]	0.037±0.002 [a]

❖ *Values are mean of triplicate determination.*

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. Result

3.1. Growth and feed conversion

The effects of vitamin-C enriched feed on the growth parameters and FCR under four treatments are summarized in Table-3. Significantly higher weight gain and SGR was recorded in T3 followed by T4, T2 and the lower weight gain and SGR was in T1 (Control). From the result, it was also observed that lower dose of dietary vitamin-C (T2) had no significance effects on weight gain and SGR compared to the control. Significantly lower FCR value was recorded in T3 and the higher was in T1 followed by T4 and T2. There was no significance difference was observed in survival rate of the fish among the treatments.

Table-3: Growth parameters and feed conversion ratio of the fish under four treatments

Parameters	Treatment			
	T1	T2	T3	T4
Initial weight (g)	210.12±1.71[a]	209.47±4.46[a]	210.33±0.50[a]	210.43±0.41[a]
Final weight (g)	534.34±13.84[c]	542.67±11.56[c]	570.56±13.36[a]	554.67±10.15[b]
Weight Gain (g)	324.22±5.13[c]	333.20±6.11[c]	360.23±4.36[a]	344.24±9.01[b]
SGR (% bwd^{-1})	1.02±0.03[c]	1.04±0.02[c]	1.12±0.03[a]	1.08±0.02[b]
SR (%)	93.60±1.83[a]	94.08±1.60[a]	94.50±1.15[a]	93.50±1.25[a]
FCR	1.91±0.05[a]	1.80±0.06[b]	1.56±0.05[c]	1.73±0.06[d]

❖ *T1 was assigned to the fish treated with Feed-1 (Control, feed without vitamin-C), T2 was assigned to the fish treated with Feed-2 (200 mg vitamin-C/kg feed), T3 was assigned to the fish treated with Feed-3 (300 mg vitamin-C/kg feed) and T4 was assigned to the fish treated with Feed-4 (400 mg vitamin-C/kg feed). Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).*

3.2. Blood parameters

The changes in blood parameters of the fish due to vitamin-C enriched feed in the current study are summarized in Table-4. Significant difference in WBC count was found between the control (T1) and the vitamin-C treated fish, but there was no significance difference among the treated fish (T2, T3 and T4). Relatively higher WBC count was recorded in T3 and T4. Lower dose of vitamin-C (200 mg/kg feed) in the feed had no significant effects on RBC count and Hb level whereas higher dose (300 and 400 mg/kg feed) had significant effects. The lower count of RBC and Hb level was recorded in T1 and the higher in T4. PCV values showed increasing trend with increasing doses of vitamin-C in the feed and significantly higher value was recorded in T4. Moreover, the MCV, MCH and MCHC values showed decreasing trend with increasing doses of vitamin-C in the feed.

Table-4: Blood parameters of the fish under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
WBC ($x10^4$ mm^{-3})	7.46±0.42[b]	8.51±0.32[a]	8.75±0.27[a]	8.83±0.31[a]
RBC ($x10^6$ mm^{-3})	2.36±0.07[c]	2.40±0.05[c]	2.53±0.09[b]	2.88±0.09[a]
Hb (g/dl)	7.15±0.15[c]	7.15±0.11[c]	7.75±0.15[b]	8.30±0.10[a]
PCV (%)	17.60±0.20[d]	21.58±0.46[c]	22.90±0.80[b]	23.90±0.50[a]
MCV (fL)	87.75±3.98[a]	71.22±1.85[b]	66.80±2.50[c]	67.10±2.20[c]
MCH (pg)	26.43±1.57[a]	25.67±1.10[a]	21.07±1.95[b]	19.35±1.87[b]
MCHC (g/dl)	30.04±1.44[a]	29.50±1.67[a]	28.70±1.70[a]	27.35±0.15[a]

❖ *T1 was assigned to the fish treated with Feed-1 (Control, feed without vitamin-C), T2 was assigned to the fish treated with Feed-2 (200 mg vitamin-C/kg feed), T3 was assigned to the fish treated with Feed-3 (300 mg vitamin-C/kg feed) and T4 was assigned to the fish treated with Feed-4 (400 mg vitamin-C/kg feed). Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).*

3.3. Carcass compositions

The changes in carcass compositions of the fish due to vitamin-C enriched feed in the current study are summarized in Table-4. There was no significant difference in the moisture, ash, carbohydrate and crude fiber contents in carcass among the treatments. An increasing trend was observed in ash, carbohydrate and crude fiber content in carcass with the increasing level of vitamin-C in the feed. Significantly higher crude protein was found in the T3 and T4 from the T1 and T2 but no significant difference between T3 and T4, and T1 and T2. The lipid content was relatively higher in all vitamin treated fish than the control fish and significantly higher lipid content was recorded in T2.

Table-4: The carcass compositions of fish flesh under four treatments

Component (% dry basis)	Treatments			
	T1	T2	T3	T4
Moisture	8.68 ± 0.27^{a}	8.63 ± 0.12^{a}	8.33 ± 0.39^{a}	8.26 ± 0.46^{a}
Crude protein	71.09 ± 0.47^{c}	72.13 ± 0.37^{b}	72.73 ± 0.41^{a}	72.59 ± 0.53^{a}
Lipid	8.27 ± 0.24^{b}	8.73 ± 0.21^{a}	8.43 ± 0.29^{b}	8.39 ± 0.19^{b}
Ash	6.62 ± 0.27^{a}	6.61 ± 0.33^{a}	6.84 ± 0.30^{a}	6.71 ± 0.23^{a}
Carbohydrate	2.58 ± 0.11^{a}	2.61 ± 0.13^{a}	2.63 ± 0.17^{a}	2.66 ± 0.24^{a}
Crude fiber	1.20 ± 0.09^{b}	1.34 ± 0.10^{a}	1.37 ± 0.11^{a}	1.41 ± 0.14^{a}

❖ *T1 was assigned to the fish treated with Feed-1 (Control, feed without vitamin-C), T2 was assigned to the fish treated with Feed-2 (200 mg vitamin-C/kg feed), T3 was assigned to the fish treated with Feed-3 (300 mg vitamin-C/kg feed) and T4 was assigned to the fish treated with Feed-4 (400 mg vitamin-C/kg feed). Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).*

IV. Discussion

4.1. Growth and feed conversion

From the result of current study, it was found that feeds prepared with different level of vitamin-C provide better growth performance and feed conversion ratio of the fish compared to the control (Feed without vitamin-C). Most studies on other species indicate that vitamin-C improved weight gain and feed conversion ratio [6][16][17] that also supported the findings of present study. Significantly higher weight gain were recorded in T3 (300 mg vitamin C/kg feed) and the lower weight gain was recorded in T1 (Control). The present findings are agreed with the finding of Nsonga *et al.* [18] and Daniel *et al.* [17] who stated that juvenile tilapia (*Oreochromis karongae*) and juveniles striped catfish (*Pangasianodon hypophthalmus*) fed the diet with ascorbic acid (AA) had significantly better growth as compared to the feed without AA. Okhionkpamwonyi and Edema [8] denoted that African catfish (*Clarias gariepinus)* fed diets containing vitamin-C had positive effects on weight gain compared with those fed the control diet. According to the result of current study, the fish in T3 showed the higher SGR which might be due to addition of vitamin-C in the feed as compared to the fish in T1 (Control). The findings of the current study are similar with the findings of Nsonga *et al.* [18] who found highest SGR in juvenile tilapia (*Oreochromis karongae*) fed with vitamin-C enriched feed. The present finding is also supported by the finding of Daniel *et al.* [17] who found highest SGR in striped catfish juveniles (*Pangasianodon hypophthalmus*) with the addition of vitamin-C in feed. Misra *et al.* [5] also stated positive effect of various levels of vitamin-C on better specific growth of *L. rohita*.

The values of FCR significantly varied among the treatments. The lower FCR value was recorded in T3 whereas the higher FCR value was recorded in T1 (Control). The findings of the present study are similar with the findings of Nsonga *et al.* [18] who found lower feed conversion ratio in juvenile tilapia (O*reochromis karongae*) fed with vitamin-C enriched feed. Shahkar *et al.* [7] observed lower FCR value for Japanese eel (*Anguilla japonica*) with the addition of 423 and 840 mg vitamin-C kg^{-1} feed which also supported the findings of the present study. In the present study, there was no significance difference in survival rate of the fish among the treatments. Comparatively lower survival rate was found in T1 and T4 where as the higher survival rate was recorded in T2 and T3. The better survival rate recorded in the present study from the reports of Misra *et al.* [5] and Okhionkpamwonyi and Edema [8] might be due to the stocking of healthy juveniles and the maintaining of better environment during the study period.

4.2. Blood parameters

Blood parameters are important health indicators which reveal the health condition of fish regarding diseases and immune system condition before and after an experiment. The changes in blood composition can be result of changes occurred in diet quality and nutrient compounds [19]. In this study, inclusion of vitamin-C in the feed showed significant effects on blood parameters between vitamin-C treated fish and control fish. According to the current study, the total WBC count increased significantly in T2, T3 and T4 compared to the T1 (Control). The findings of the present study are similar with the findings of Nsonga *et al.* [18] and Miar *et al.* [6] who stated increased WBC count in juvenile tilapia (*Oreochromis karongae*) and rainbow trout (*Oncorhynchus mykiss*) fed

with vitamin-C enriched feed. This result is also supported by Shahkar *et al*. [7] who denoted that the total WBC count increased with the addition of vitamin-C in feed of Japanese eel (*Anguilla japonica*). The present finding is also more or less similar with the finding of Okhionkpamwonyi and Edema [8] who observed increased WBC count in African Catfish (*Clarias gariepinus)* with the addition of vitamin-C in feed.

The red blood cells, PVC (haematocrit) and hemoglobin of fish body are responsible for carrying oxygen. In the current study, RBC and PVC values were increased significantly whereas Hb levels increase less significantly in vitamin-C treated fish compared to the control fish. From the result it was observed that dietary vitamin-C in *L. rohita* 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 Okhionkpamwonyi and Edema [8] who cited that red blood cells counts, haematocrit and hemoglobin of African catfish (*Clarias gariepinus)* were significantly higher in all treatments receiving vitamin-C supplemented-diets. Suggestion reason for this influence may be attributed to the lowered stress and healthy environment in fish that cause improve immune system function as well as metabolic activity. Moreover, vitamin-C inclusion in the feeds had considerable effects on MCH, MCHC and MCV values in the treated fish compared to the control fish. Most studies on other species indicate that dietary vitamin-C had considerable positive effects on blood parameters [6][7][18] those also supported the findings of the present study.

4.3. Carcass compositions

According to the current study, the crude protein content in carcass was significantly increased in vitamin-C treated fish. The significant improvement of carcass crude protein of the fish indicated the importance of vitamin-C in body protein metabolism. Such improvement was also observed in *Cyprinus carpio* [20] and in *Barbus sharpeyi* [21]. Vitamin-C is an essential coenzyme in certain oxidative process, including the oxidation tyrosine and phenylalanine [22]. From the results it was also observed that lower dose of vitamin-C had more positive effects on crude lipid content in the carcass of the fish which might be due to the effects of dietary vitamin-C in certain level on lipid metabolism. Moreover, vitamin-C inclusion in the commercial feed had no significance effects on ash, carbohydrate and crude fiber content of carcass of *L. rohita* in the current study but an increasing trend was observed with increasing concentration of vitamin-C in the feed. These results revealed that dietary vitamin-C has positive role in mineral metabolism and deposition.

V. Conclusion

The present study demonstrated that inclusion of vitamin-C in the feed at 300 mg/kg in cage culture system has better effects on the growth, blood indices and carcass compositions of *L. rohita*. Long term studies are required to draw more accurate conclusion on the effects of vitamin-C enriched feed on the growth, blood parameters and carcass composition of the fish.

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