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Effect of dietary vitamin C on the growth and survival rate of rohu (*Labeo rohita*)

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ABSTRACT

The experiment was carried out to evaluate the effect of dietary vitamin C on the growth and survival rate of rohu (Labeo rohita) for a period of 49 days from 5th April to 24th May, 2016 in 12 experimental aquaria in Wet Laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. Fingerlings of initial weight of 6.02±0.00 g was released at the same stocking density (12 fingerlings per aquaria). Four different treatments (T_1, T_2, T_3 and T_4) each with three replications were used having different amount of vitamin C, T_1 (0 mg vitamin C/kg feed), T_2 (200 mg vitamin C/kg feed), T_3 (400 mg vitamin C/kg feed) and T_4 (600 mg vitamin C/kg feed). All four diets having a constant inclusion level of the following ingredients: fish meal 40%, rice bran 25%, wheat bran 25%, molasses 5%, soybean oil 4% and vitamin and mineral premix 1%. Final weight (g), weight gain (g), percent weight gain (%), specific growth rate (%/day), and protein efficiency ratio, varied from 7.31±0.14 to 7.83±0.15, 1.29±0.14 to 1.8±0.15, 21.48±2.37 to 30.12±2.54, 0.52±0.22 to 1.21±0.17 and 0.46±0.42 to 0.60±0.04, respectively. Highest FCR (7.81±0.22) was found in T_1 and the lowest FCR (5.78±0.36) was found in T_4. The highest PER (0.60±0.04) was found in T_4 and the lowest PER (0.46±0.42) was found in T_1. The highest survival rate (63.89±4.81) was found in T_4 and lowest survival rate (47.22±9.62) was found in T_1. Best growth performance was found in T_4 followed by T_3, T_2 and T_1. The present research findings suggested that the best growth performance and survival rate of Labeo rohita was obtained from supplementation of 600 mg vitamin C per kg feed containing diet which could be chosen by the fish feed manufacturer and fish farmers.

Key Words: *Vitamin C, Labeo rohita, Growth and Survivality.*

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I. Introduction

Aquaculture plays a vital role in many countries by offering better nutrition, higher income, earning foreign exchange and better employment opportunities. World per capita apparent fish consumption

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increased from an average of 9.9 kg in the 1960's to 19.2 kg in 2012 (FAO, 2014). According to FAO (2014), aquaculture is the fastest growing sector of the world's animal production with an annual increase of about 3.2%. To sustain such high rates of fish production, a matching increase in fish feed production is imperative. Among the farmed species Indian major carp, *Labeo rohita* is one of the most important and popular fish species considering its taste and market demand. But feed cost is the major constraint for the culture of tilapia and other culturarable species. Many works have been done on feed formulation (Hossain and Hasan, 2000; Habib *et al.*, 2001; Dewan *et al.*, 2004) but high cost and fluctuating quality as well as the uncertain availability of fish meal have led to the need to add additional ingredients for fish feed formulation which may lead to enhance fish production. To formulate cost effective feed we should use different but essential feed ingredients.

Vitamins are one of the food components which are very important in diet in spite of their low amounts. Vitamin C is an essential vitamin for normal physiological functions in animals including fish (Lim and Lovell, 1978). Most teleosts are unable to synthesize ascorbic acid due to the lack of l-gulonolactone oxidase (EC 1.1.3.8) that is responsible for synthesis of vitamin C (Fracalossi *et al.*, 2001). Therefore, an exogenous source of vitamin C is required in fish diets. Inadequate supply of dietary vitamin C usually results in a number of deficiency signs such as spinal deformation, impaired collagen formation, internal haemorrhaging and retarded growth (Gouillou-Coustans *et al.*, 1998). The quantitative requirements for dietary vitamin C have been determined for several fish species and recommended values ranged from 20 to 50 mg ascorbic acid per kg diet (NRC, 1993). More than 400 mg/kg diet is needed for better growth of rohu (Gao *et al.*, 2013). Misra *et al.* (2007)suggested that more than 800 mg/kg vitamin C needed for better growth and survival rate of common carp. The amount of vitamin C required in the diet for growth is from 30 to 90 mg kg-' of diet (Lovell, 1973; Andrews and Murai, 1975). The requirement of vitamin C varies, to some degree, with fish species, size, diet and experimental conditions.The present study was designed to evaluate the growth performances of rohu by feeding the feed formulated containing vitamin C which would be helpful the rural people in uplifting their nutrition, livelihood and additional income.

II. Materials and Methods

Experimental site: The proposed research work were undertaken in 12 glass aquaria (each contains 60L) at the Wet Laboratory, adjacent to the Faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. Stored ground water was used for rearing the fish. Aeration was provided from a 1.0-HP compressor to all the experimental tanks.

Experimental Design: The experiment was conducted in 12 glass aquaria considering four treatments [Treatment 1 (T_1), Treatment 2 (T_2) and Treatment 3 (T_3), Treatment 4 (T_4),] each having three replications. In this experiment Diet 1: 0 mg vitamin C, Diet 2:200 mg vitamin C; Diet 3:400 mg vitamin C, Diet 4:600 mg vitamin C were used for the treatment 1, 2,3 and 4, respectively. The *Labeo rohita* fry was randomly distributed at a rate of 12 fish per aquaria. All the aquaria were kept on 1 m high wooden table to facilitate better observation and accessibility.

Sample collection and acclimation: Fingerlings of *Labeo rohita* were collected from the fish seed production hatchery, adjacent area of Mymensingh district. Rohu fry hatched from single egg mass were used in this experiment and acclimated to laboratory condition in two glass aquarium (120 L capacity each) for 2 weeks by feeding a commercial carp diet.

Feed ingredients collection and feed formulation: The selected ingredients for this experiment-fish meal, rice bran, wheat bran, molasses, Vitamin C; mineral and vitamin premix were purchased from local market. The formula and analyzed proximate composition of the basal diet is shown in Table 01. Four graded levels of vitamin C (L-ascorbic acid, AA) at 0, 200, 400, and 600 mg/Kg diets were included in the basal diet (AA will be supplemented separately to the basal diet at the expense of wheat flour). The ingredients were grinded, milled, weighed, mixed and pelleted with meat mincer through a 0.8 mm diameter. After pelleting, the feeds were air dried and put in an air-tight container. All diets were stored at -20°C until fed.

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Table 01. Formulation of the basal diet (dry matter basis)

Sl No	Ingredients	%	Sl No	Ingredients	%
1.	Fish meal	40	4.	Molasses	5
2.	Rice bran	25	5.	Soybean oil	4
3.	Wheat bran	25	6.	Mineral Premix	1

Table 02. Proximate composition analysis of different feed ingredients (dry basis)

Feed ingredients	Crude protein (%)	Crude lipid (%)	Moisture (%)	Ash (%)	Crude fibre(%)	NFE (%)
FM	55.31	13.71	8.87	16.57	2.70	2.84
RB	12.46	14.18	13.71	11.47	10.78	37.40
WB	14.51	4.03	14.46	6.24	15.26	45.50

Table 03. Proximate composition analysis of formulated feed (dry basis)

List of diet	Moisture(%)	Crude Lipid (%)	Crude Protein(%)	Ash (%)	Crude Fiber(%)	NFE (%)
Diet I	10.45	7.40	27.90	14.60	5.40	34.25
Diet II	10.59	7.95	28.40	14.90	4.90	33.26
Diet III	10.35	8.20	28.65	15.40	5.80	31.6
Diet IV	10.09	7.90	28.80	14.60	5.50	33.11

Experiment procedure: The experiment was conducted for 70 days. Each treatment had three replications. The fingerlings of *Labeo rohita* initial were of 6.02 gm weight. The fingerlings were randomly distributed at a rate of 12 fish per aquaria. Feeding was done twice daily at 9.00 am and 5.00 pm. Partial change of water from each aquarium was done daily during the removal of feces and uneaten feed.

Feeding rates: Fingerlings were fed with experimental diets twice daily in the morning at 9.00 am and in the afternoon at 5.00 pm throughout the study period. Fingerlings in each aquarium were fed daily at the rate of 10% of their body weight; the amount was fixed after observing that it was not interested to take more than that amount of feed.

Sampling procedure: Initial and final weight of fish in each aquarium was recorded. Fish were bulk weighed at every seven (7) days interval to keep record of fish weight. Fish were netted by using a fine mesh scoop net and excess water was then removed from fish body gently by using a blotting paper before weighing to the digital balance. After weighing of the fingerlings, those were released in the aquarium.

Analytical methods: Proximate composition of prepared feeds and individual ingredients were analyzed in the Nutrition Laboratory of the Department of Aquaculture, BAU following Association of Official Analytical Chemists (AOAC, 2000) methods with slight modifications as below:

$$\text{Moisture (\%)} = \frac{\text{Original sample weight (g)} - \text{Dried sample weight (g)}}{\text{Original sample weight (g)}} \times 100$$

$$\text{Ash content (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude protein

$$\text{\% Nitrogen} = \frac{\text{Milliequivalent of nitrogen (0.014) x titrant value (ml) x strength of HCL}}{\text{Sample weight (g)}} \times 100$$

Crude Protein (%) = 6.25 x % Nitrogen, for animal source
Crude Protein (%) = 5.87 x % Nitrogen, for plant source

Crude lipid (%) = (extracted lipid/ sample weight) × 100

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$$\text{Crude fibre (\%)} = \frac{\text{Wt of sample after air drying (g) - Wt. of sample after ashing (g)}}{\text{Sample weight(g)}} \times 100$$

Growth Performances

Weight gain (g):Weight gained refers to as the difference between final weight and initial weight. The formula:

Weight gain (g) = mean final weight (g) – mean initial weight (g)

Percent weight gain: $= (W_2 - W_1 / W_1) \times 100$

Where, W_1 = the mean initial fish weight

W_2 = the mean final fish weight.

Specific growth rate (% /day) =

$$\frac{\text{Ln } W_2 - \text{Ln } W_1}{T_2 - T_1} \times 100$$

Where, W_2 = Final live body weight (g) at time T_2

W_1 = Initial live body weight (g) at time T_1

T_2-T_1 = No. of days of the experiment

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed fed (dry weight)}}{\text{Live weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Live weight gain (g)}}{\text{Crude protein fed (g)}}$$

Nitrogen free extracts (NFE) (%) = {100- (moisture + crude protein + crude lipid + ash + crude fibre)}

$$\text{Survival rate (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number of fish stocked}} \times 100$$

Data analysis: The collected data were statistically analyzed by one way ANOVA with the help of SPSS to see whether the influence of different treatments on these parameters were significant or not. The means of different treatment were compared by Duncan's New Multiple Range Test (Duncan, 1955) to test the significance of variation between the treatment means. Standard error (SE) of the treatment means was calculated from the residual mean square in the analysis of variance. All statistical analyses were carried out by MS Excel 2000 (version 7.0).

III. Results and Discussion

Effects of different diet such as Diet I: vitamin C (0 mg), Diet II: vitamin C (200 mg), Diet III: vitamin C (400 mg), Diet IV: vitamin C (600 mg) is discussed below:

Growth performance of *Labeo rohita*

The growth performance of *Labeo rohita* in terms of initial weight (g), final weight (g), weight gain (g), percent weight gain, specific growth rate (%/day) were calculated at the end of the experiment.

Initial weight (g)

The initial average weight of *Labeo rohita* in different treatments was 6.02g (Table 05).

Final weight (g)

The mean final weight of *Labeo rohita* in different treatments varied from 7.31 g to 7.83 g (Table 05). The mean weight gain (g) in treatment T_4 was found highest and followed by T_3, T_2 and T_1, respectively (Figure 01).

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Weight gain (g)
The mean weight gain of different treatments ranged from 1.29 g to 1.81 g (Table 05). The mean weight gain of experimental fish was found highest in treatment T_4 followed by T_3, T_2 and T_1, respectively (Figure 02).

Percent weight gain (%)
The percent weight gain of fish in different treatments ranged from 21.48% to 30.12% (Table 05). The highest percent weight gain (%) was found in treatment T_4followed by T_3, T_2 and T_1, respectively. There was significant ($P<0.05$) variation of percent weight gain between T_1 and T_4; T_2 and T_4 but no significant variation between T_3 and T_2; T_3 and T_1; T_3 and T_4 (Figure 03).

Specific growth rate (%/day)
The specific growth rate (%/day) ranged from 0.52% to 1.21%/day (Table 7). The highest specific growth rate (1.21%/day) was found in $T_4$and followed by T_3, T_2 and T_1, respectively. There was significant ($P<0.05$) variation of specific growth rate (%/day) between T_1 and T_4; T_2 and T_4 but no significant variation between T_3 and T_2; T_3 and T_1; T_3 and T_4 (Figure 04).

Food conversion ratio (FCR)
Mean food conversion ratio (FCR) in different treatments was ranged from 5.78 to 7.81(Table 05). The highest FCR value was obtained in treatment T_1 followed by T_2,T_3, and T_4, respectively. There was significant ($P<0.05$) variation in mean food conversion ratio (FCR) between T_1 and T_4; T_2 and T_4 but no significant variation between T_3 and T_2; T_3 and T_1; T_3 and T_4 (Figure 05).

Food conversion efficiency
Mean food conversion efficiency (FCE) in different treatments was varied from 0.13 to 0.17 (Table 05). The highest FCE value was obtained in treatment T_4 and followed by T_3, T_2, and T_1, respectively. There was significant ($P<0.05$) differencebetweenT_1 and T_4; $T_2$and T_4; T_3 and T_4but no significant variation between T_1 and T_2; T_2 and T_3 (Figure 06).

Protein efficiency ratio (PER)
Mean protein efficiency ratio (PER) in different treatments varied from 0.46 to 0.60 (Table 05).The highest PER value was obtained in treatment T_4 and followed by T_3,T_2, and T_1, respectively. There was significant ($P<0.05$) differencebetweenT_1 and T_4; T_2 and T_4 but no significant variation between T_3 and T_2; T_3 and T_1; T_3 andT_4; T_1 and T_2 (Figure 07).

Survival rate (%)
After 49 days rearing period the mean survival rate (%) of *Labeo rohita* under different treatments ranged from 47.22% to 63.89% (Table 05). There was significant ($P<0.05$) variation between the treatments T_1, andT_4 but no significant variation between T_1 and T_2; T_3 and T_1; T_3 and T_2 (Figure 08).

Table 04. Mean (±SD) weight increment (g) of *Labeo rohita* in different treatments at 7 days interval during the experimental period

Sampling date	Average Weight (g)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Initial sampling	6.02±0.00	6.02±0.00	6.02±0.00	6.02±0.00
1st week	6.19±0.181	6.16±0.125	6.14±0.276	6.24±0.202
2nd week	6.39±0.206	6.40±0.243	6.31±0.392	6.53±0.301
3rd week	6.57±0.176	6.62±0.197	6.55±0.308	6.79±0.192
4th week	6.75±0.169	6.83±0.177	6.81±0.209	7.05±0.201
5th week	6.97±0.171	7.04±0.165	7.05±0.205	7.35±0.205
6th week	7.14±0.154	7.25±0.152	7.29±0.203	7.57±0.152
Final sampling	7.31±0.143	7.46±0.146	7.53±0.206	7.83±0.153

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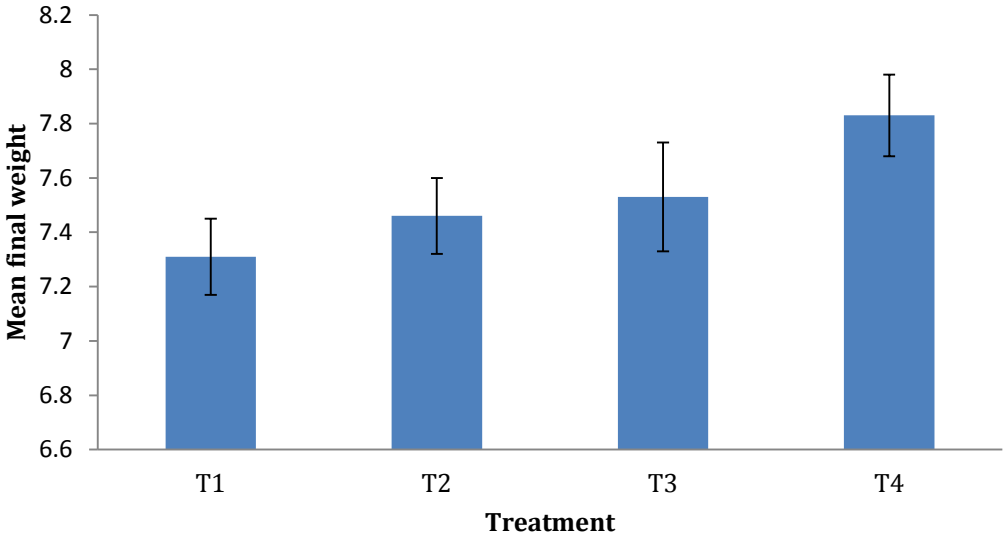

Figure 01. Mean final weight (g) of *Labeo rohita* in different treatments during the experimental period.

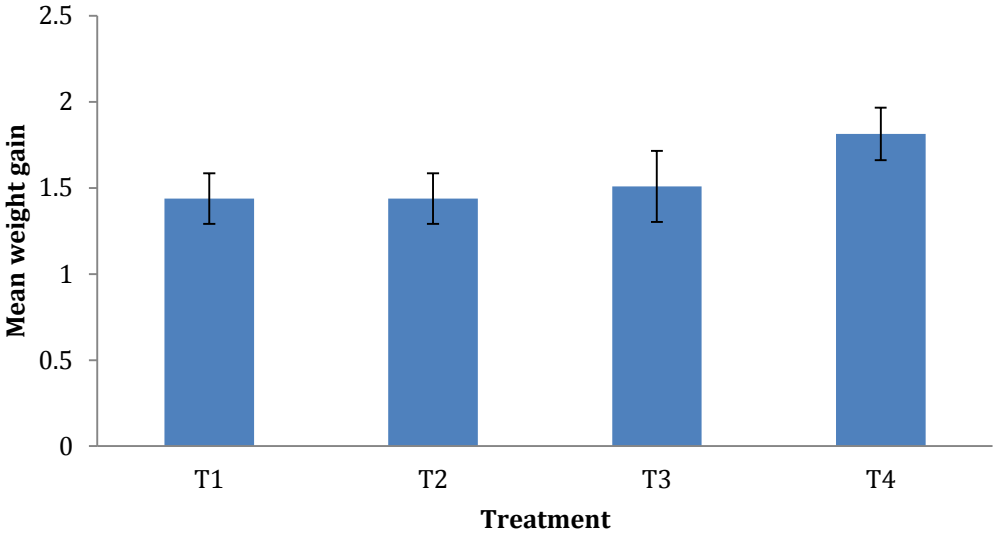

Figure 02. Mean weight gain (g) of *Labeo rohita* in different treatments during the experimental period.

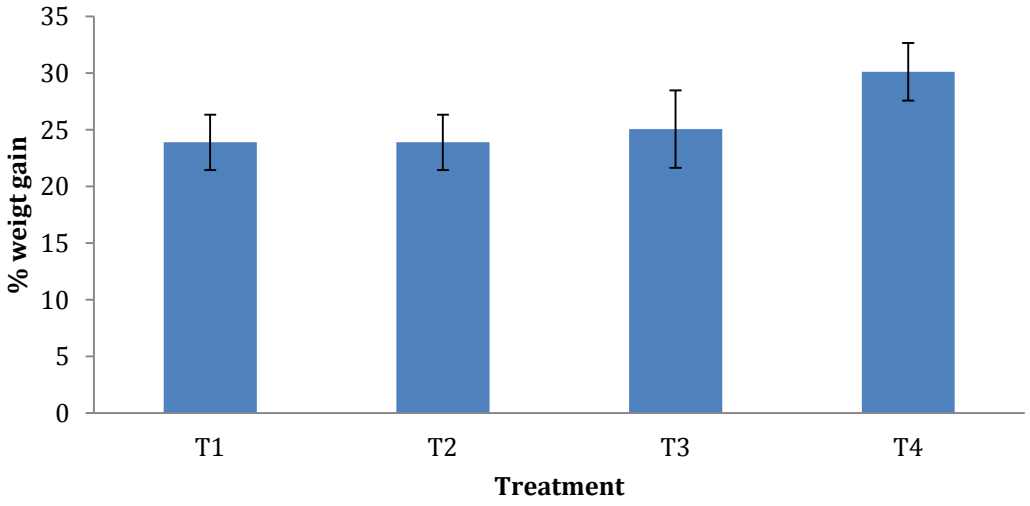

Figure 03. Mean percent weight gain (%) of *Labeo rohita* in different treatment during experimental period.

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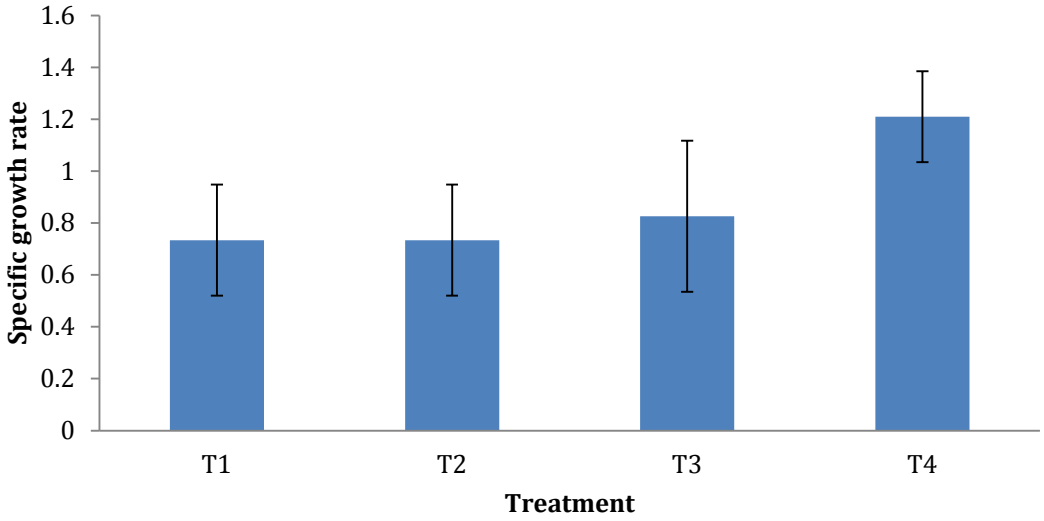

Figure 04. Mean specific growth rate (%/day) of *Labeo rohita* in different treatments during the experimental period.

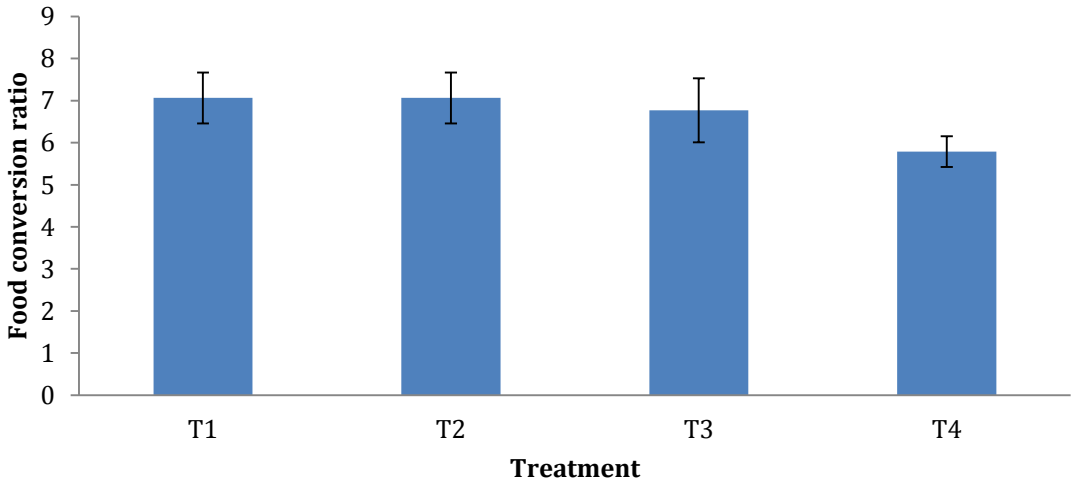

Figure 05. Mean Food conversion ratio of *Labeo rohita* in different treatmentsduring the experimental period.

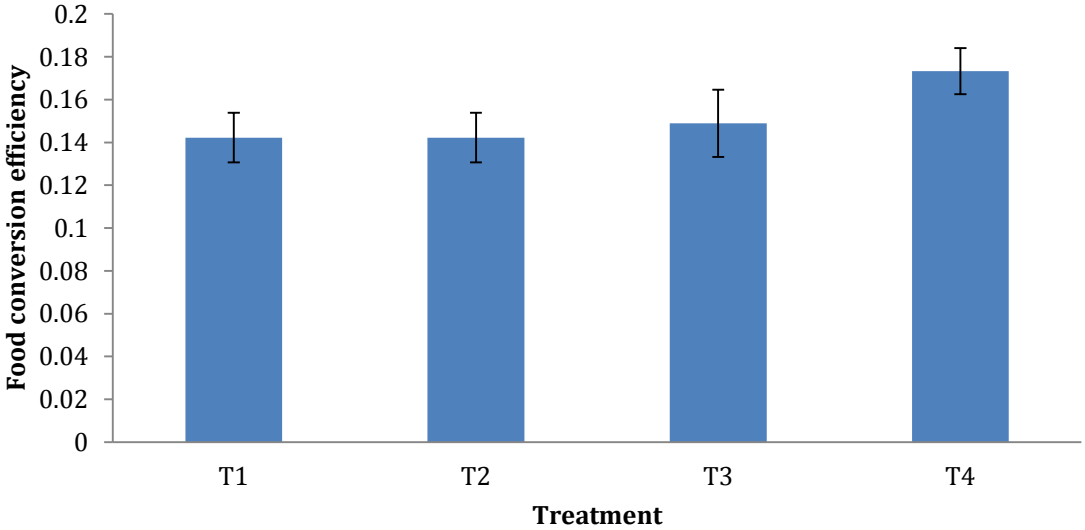

Figure 06. Mean Food conversion efficiency of *Labeo rohita* in different treatments during the experimental period.

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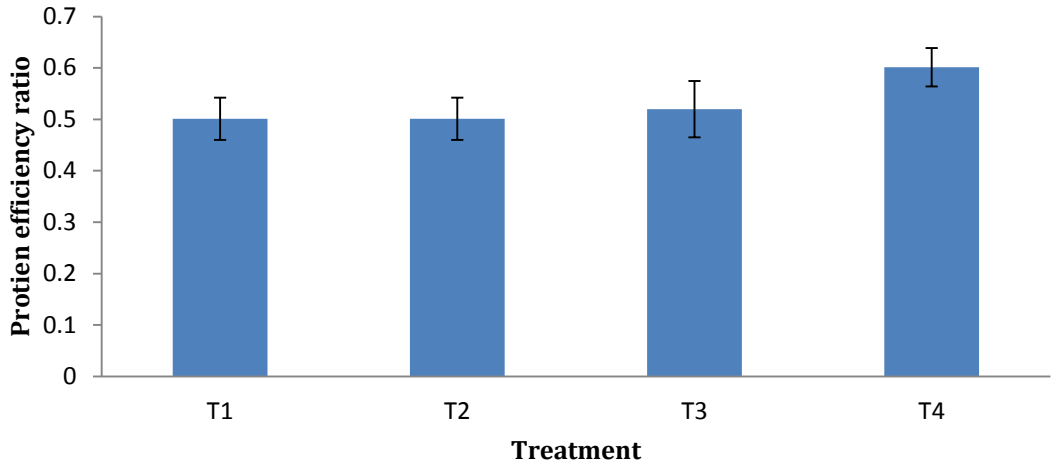

Figure 07. Mean Protien efficiency ratio of *Labeo rohita* in different treatments during the experimental period.

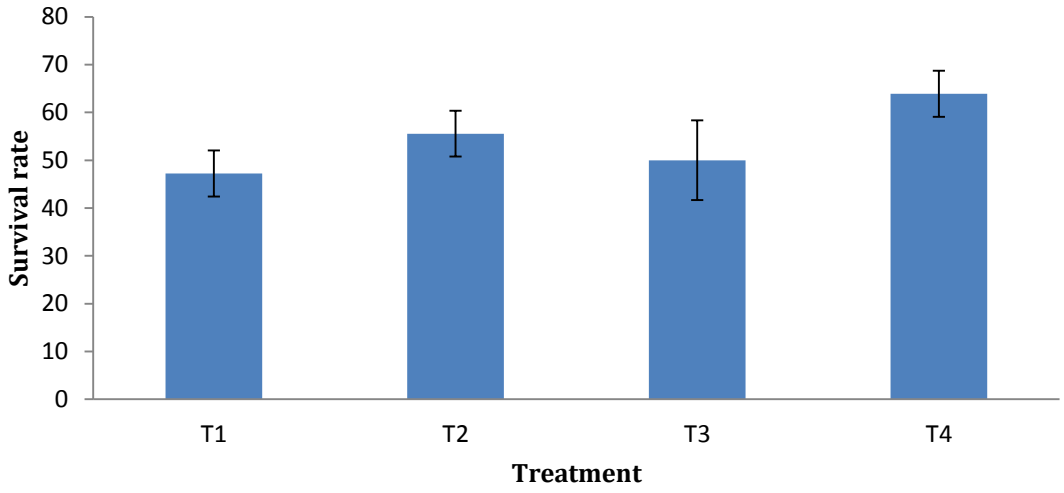

Figure 08. Mean survival rate (%) of *Labeo rohita* in different treatments during the experimental period.

Table 05. The effect of different treatments on growth performance, feed utilization and survival of *Labeo rohita* reared in aquarium (Mean ± SE) during the study period

Variable/ Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Initial weight (g)	6.02±0.00	6.02±0.00	6.02±0.00	6.02±0.00
Final weight (g)	7.31(±0.14)^{a}	7.46(±0.14)^{a}	7.53(±0.20)^{ab}	7.83(±0.15)^{b}
Weight gain (g)	1.29(±0.14)^{a}	1.44(±0.15)^{a}	1.51(±0.21)^{ab}	1.81(±0.15)^{b}
% weight gain	21.48(±2.37)^{a}	23.89(±2.43)^{a}	25.06(±3.42)^{ab}	30.12(±2.54)^{b}
SGR (%/day)	0.52(±0.22)^{a}	0.73(±0.21)^{a}	0.83(±0.29)^{ab}	1.21(±0.17)^{b}
FCR	7.81(±0.69)^{b}	7.06(±0.60)^{b}	6.76(±0.76)^{ab}	5.78(±0.36)^{a}
FCE	0.13(±0.01)^{a}	0.14(±0.01)^{a}	0.15(±0.01)^{a}	0.17(±0.01)^{b}
Survival rate (%)	47.22(±9.62)^{a}	55.55(±4.81)^{ab}	50(±8.33)^{ab}	63.89(±4.81)^{b}
PER	0.46(±0.42)^{a}	0.50(±0.04)^{a}	0.51(±0.05)^{ab}	0.60(±0.04)^{b}

Values given in bracket are standard deviation. The values in the same row having similar letter (s) do not differ significantly otherwise differ significantly ($p<0.05$) as per Duncan Multiple Range Test (DMRT).

Growth performance of rohu (*Labeo rohita*)

In this experiment effect of dietary supplementation of different levels of vitamin C on the growth performance of rohu (*Labeo rohita*) fingerlings in aquarium were investigated. Compared with other treatments growth performance of rohu fingerlings were significantly high in treatment 4 which were

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provided with higher levels of vitamin C (600 mg/kg) feed whereas lower in $T_1$and other feed provided with vitamin C (200 and 400 mg/kg). Tewary and Patra (2008) assessed the effects of different dietary levels vitamin C on the growth performance of rohu fingerlings at a concentration of 500, 1000 and 1500 mg/kg diet for 60 days and recommended that vitamin C concentration up to 1000 mg/kg diet was the most appropriate concentration for the better growth of rohu fingerlings. In our study, the maximum mean final weight was 1.813±0.1530 g in treatment 4, whereas, the minimum mean final weight was 1.293±0.1428 g in T_1. Nsonga *et al.* (2009) showed that the maximum mean final weight in juvenile tilapia (*Oreochromis karongae*) was 13.79±.028 g where vitamin C 60 mg/kg feed was supplied and minimum mean final weight was 4.78±0.12g where no vitamin C provided in feed.

On the other hand, Misra *et al.* (2007) evaluated the effects of different dietary levels of vitamin C on the immunity, growth and survival Indian major carp *Labeo rohita*. A 8th week feeding trial was conducted to assess the effect of vitamin C where Diet 1: 0 mg/kg, Diet 2: 100 mg/kg, Diet 3: 200 mg/kg and Diet 4: 500 mg/kg vitamin C was supplemented in the feed. Results showed that supplementation of vitamin C 500 mg/kg feed had significant positive effects on the food conversion ratio (FCR), specific growth rate (SGR), average daily gain (ADG) and recommended that Diet 4 containing 500 mg/kg vitamin C feed had been found to be more effective for better growth of *Labeo rohita*.

In the present study, the food conversion ratio (FCR) in treatment 1 (control) aquarium was 7.81±0.69, treatment 2 was 7.06±0.60, treatment 3 was 6.76±0.76 and treatment 4 was 5.78±0.36. Here food conversion ratio was ranged from 5.78 to 7.81. The lowest food conversion ratio was observed in treatment 4. Tewary and Patra (2008) reported that the food conversion ratio (6.10±0.18%) was found in the control (without vitamin C) while the lowest food conversion ratio (3.43±0.19%) was measured in treatment 2 (1000 mg vitamin C per kg feed).

Food conversion efficiency ranges from 0.13±0.01 to 0.17±0.01. Highest food conversion efficiency was found in treatment 4 and lowest in treatment 1. Faramarzi (2012) experimented that food conversion efficiency in common carp (*Cyprinus carpio*) was 0.37±0.01 to 0.38±0.02. Maximum food conversion efficiency was found in treatment 4 which feed contains 2000 mg vitamin C and minimum in control without vitamin C.

Protein efficiency ratio in treatment 1 aquarium was 0.46±0.42, treatment 2 was 0.50±0.04, treatment 3 was 0.51±0.05 and treatment 4 was 0.60±0.04. The lowest protein efficiency ratio was observed in treatment 1 and highest in treatment 4. Gbadamosi *et al.* (2006) found the highest protein efficiency ratio in African catfish (*Clarias gariepinus*) 1.08±0.02 in treatment 4 where vitamin C was 200 mg/kg feed and lowest protein efficiency ratio 0.55±0.01 in control where feed supplied without vitamin C.

The survival rate in treatment 1 (control) aquarium was 47.22±9.62, treatment 2 was 55.55±4.81, treatment 3 was 50±8.33 and treatment 4 was 63.89±4.81%. In the present study survival rate varied from 47.22±9.62 to 63.89±4.81%. Survival rate was higher in treatment 4 where the vitamin C level (600 mg/kg feed) and lower in treatment 1 (control) aquarium where fish reared without vitamin C. Ashraf and Rauf (2008) observed that 70±6.0% survival rate in treatment 3 where the vitamin C level (300 mg/kg feed) and lowest in control 30±6.0% where no vitamin C was supplemented.

The specific growth rate (SGR%/day) in treatment 1 (control) was 0.52±0.22, treatment 2 was 0.73±0.21, treatment 3 was 0.83±0.29 and treatment 4 was 1.21±0.17% per day. In the present study specific growth rate (SGR) varied from 0.52±0.22 to 1.21±0.17% per day. SGR value was higher in treatment 4 where the vitamin C level was higher (600 mg/kg feed) and SGR value was lower in treatment 1 (control) aquarium. Alam *et al.* (2009) found the highest value of SGR of *Heteropneustes fossilis*in treatment 2 (1200 mg/kg feed) 1.75±0.10% per day and lowest for control (without vitamin C), 0.81±0.016% per day.

IV. Conclusion

The experiment on "Effect of dietary vitamin C on the growth and survival rate of rohu (*Labeo rohita*)" fingerlings reared in aquarium was conducted in which feeding was done twice a day with different vitamin C levels. The best growth rate was obtained at supplementation of 600 mg vitamin C per kg

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feed which was the highest in vitamin C level content. The experiment revealed that the best growth performance and survival rate of rohu (*Labeo rohita*) fingerlings were obtained with supplementation of 600 mg vitamin C per kg feed. Further research may be conducted by increasing vitamin C levels than supplementation of 600 mg vitamin C/kg feed to observe the growth and survival of rohu fingerlings to find out the optimum level of vitamin C per kg feed in future which could be chosen by the fish farmers and feed manufacturers.

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