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Dietary vitamin C supplementation on growth and haemato-biochemical parameters of *Labeo rohita* (Ham.) fingerlings

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Abstract
The cyprinid fish *Labeo rohita* was fed with vitamin C supplemented diet at varying dosage of no vitamin C (control), 500 mg kg^{-1} (T_1), 1000 mg kg^{-1} (T_2) and 1500 mg kg^{-1} (T_3) diets for 45 days. Growth (Specific growth rate), hematological parameters (red blood cell (RBC), white blood cells (WBC), packed cell volume (PCV) and hemoglobin), biochemical (blood glucose, total protein) were evaluated during experimental period on 15th, 30th, 45th days and challenge study was conducted with *Aeromonas hydrophila* bacteria after 45th day and the samples were collected on 60th day. The group T_2 had significantly higher RBC count on all the days of sampling followed by T_1. WBC count were significantly (p<0.05) higher in T_2 group in all the days of sampling followed by T_1 and T_3. The PCV level in fish fed with supplemented diet containing vitamin C was insignificant (p<0.05) in T_2 and T_1 group up to 45th day, while group T_2 showed increasing trend and was significant on 60th day. The hemoglobin content were significantly (p<0.05) higher on 30th, 45th and 60th day in T_2 group. Blood glucose level in T_2 group showed significantly (p<0.05) higher value on the 15th day to 45th day. Protein level in T_2 was significantly higher in all the days whereas, T_2 was significant (p<0.05) on the 15th and 45th day. Vitamin C supplemented diet showed increasing specific growth rate than the control. The concentration 500 to 1000 mg kg $^{-1}$ vitamin C was found to be optimum to increase haemato-biochemical parameter and growth in *L. rohita* under the present experimental conditions. The present results suggest that the dosage 1000 mg kg^{-1} vitamin C has beneficial effect on growth haematobiochemical parameter of *L. rohita*.

Keywords: vitamin C, labeo rohita, haemato-biochemical parameter, aeromonas hydrophila

Introduction
The northeast region of India, comprised of the states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura is blessed with bounty of fisheries resources. Various reasons have been sited for slow growth of cultured species particularly Indian Major Carps in this region, which includes stressful culture environment (acidic soil and water, excess iron in water and soil), quality seed and feed, high stocking density, improper feeding and occurrence of diseases, In addition, there is a common perception about the slow growth of *Labeo rohita* in culture ponds in northeast india.

Most animal can synthesize vitamin C in sufficient quantity for normal growth and function, but a few, such as primates, guinea pigs, some birds, and many fishes, cannot because they lack the enzyme L-gulonolactone oxidase for synthesis of vitamin C from glucose (Dabrowski, 1990) [5]. Vitamin C is an essential nutrient for normal growth and physiological function of most aquatic animal species (Lim and Lovell, 1978) [16]. Supplementation of dietary ascorbic acids is essential for a variety of biological and physiological functions including increased disease resistance and wound healing (Halver, 2002) [13], improved tolerance to environmental stressors (Gapasin *et al*. 1998) [11] as well as regulation of collagen synthesis (Dabrowski, 1992) [6].

The immunomodulaory effects of dietary vitamin C has already been established in several fish spices but not hitherto in Indian major carps (Nayak *et al.* 2007) [21]. Subsequently, several Indian researchers have investigated the role of vitamin C as an immunostimulant in *L. rohita* and documented it scientifically, but no established scientific information is available from the north east region which comes under a completely different agro-climatic region (Eastern Himalayan Region). In this backdrop study was designed to examine the effect of dietary levels of vitamin C on the growth performance and some biochemical and haematological

parameters in *L. rohita.* The challenge study was also planned to adjudge the effect of dietary vitamin C on the *A. hydrophila* infected fish.

Materials and Method

Experimental Setup

The experiment was carried out at the laboratory of Fish Health and Environment, College of Fisheries, Central agricultural University, Tripura, India.

Vitamin C

L-ascorbic acid manufactured by Qualigens fine chemicals, GlaxoSmithKline Pharmaceuticals, Mumbai, India was used as the source vitamin C in the experiment.

Experimental animal

600 nos. apparently healthy *L. rohita* fingerlings (length 9.75 ± 0.8 cm and weight 16.25 ± 0.5 gm) were allowed to acclimatize in the laboratory conditions for 15 days and then used for the experiments. Fishes were provided with adequate aeration and fed with normal feed (Rice bran and mustard oil cake 1:1) @ 3% of the body weight twice a day.

Experimental diet

The experimental diet was formulated using locally available ingredients mustard oil cake, rice bran, fish meal, wheat flour & cod liver oil

Table 1: The experimental diet was formulated using locally available ingredients

Ingredients	Concentrations
Fish meal	30%
Mustard oil cake	30%
Rice bran	20.5%
Wheat flour	15.5%
Vitamin & mineral premix	2%
Cod liver oil	2%

Initially all ingredients were mixed thoroughly by adding water, and then made into pellets by using a hand pelletizer (Misra *et al.*, 2007) [20] and then dried at 40 ^{0}C for 12 hours. The dried pellets were kept in an air sealed container and stored in cool dry place for further use. Vitamin C (L-Ascorbic acid) was used at the level of 500, 1000 and 1500 mg kg^{-1} in experimental feed and named as treatment 1, treatment 2 and treatment 3, respectively. The control diet was not supplemented with vitamin C. For final experimental diets pellets were coated with appropriate concentration of vitamin C and vegetable oil by spraying. The vitamin C coated experimental pellets were stored in an air tight plastic container in refrigerator (4 ^{0}C) for further use.

Experimental design

The experiment was performed in 500l FRP tanks. The fishes were divided into four groups and each group was maintained in triplicate set containing 50 nos. of fishes. Fishes were provided with adequate aeration and fed @3% of the body weight twice a day in two equal quantities in the 6 am morning and 6 pm evening. The water was replenished approximately 50% daily during the experiment

Sampling schedule

The experimental trial was conducted for 45 days and the sampling for various parameters *viz.* growth, hematological and biochemical were carried out at definite time interval. The first sampling was done on 0 day of the stocking and subsequent sampling was done after 15 days, 30 days and 45 days of feeding trials. For each sampling 7- 9 fishes were selected randomly from each tank and analyzed for various parameters.

Physico-chemical water parameter

Water quality parameter i.e. temperature, pH, dissolved oxygen, alkalinity, Ammonia- Nitrogen (NH_3-N) were recorded at seven days intervals during the experimental period. Dissolved oxygen and Ammonia- Nitrogen (NH_3-N) were estimated by Winkler's Method and Direct Nesslerization Method, respectively. Temperature and pH were measured using Mercury in glass thermometer and pH meter respectively. Alkalinity was estimated by using standard methods (APHA, AWWA, WPCF, 1989) [4].

Proximate analysis of experimental feed

Estimation of moisture and ash content were done following the routine method. While, Estimation of protein content of the experimental feed was done by routine Kjeldahl method and fat content was determined by Soxhlet method.

Table 2: Proximate composition of experimental feed

	Moisture (%)	Protein (g 100 g^{-1})	Carbohydrate (g 100 g^{-1})	Fat (g 100 g^{-1})	Ash(g 100 g^{-1})
Control	8.13	33.25	29.69	8.25	20.68
T_1	8.10	33.87	28.98	8.35	20.70
T_2	8.18	33.25	29.29	8.65	20.77
T_3	8.11	31.12	31.32	8.65	20.80

Estimation of growth parameter

The initial and final weights of fish in each group were measured individually. Specific growth rates (SGRs) was calculated according to Laird and Needham (1988) as follows:

$$\text{SGR (\%)} = \frac{\ln [\text{final mean body weight (g)}] - \ln [\text{initial mean body weight (g)}]}{\text{Time interval (days)} \times 100}$$

Table 3: Specific growth rate (SGR) of fishes after 45 days of feeding trial with the diet containing vitamin C

Parameters	Control	T_1	T_2	T_3
Initial length (cm)	9.75 ± 0.8	9.75 ± 0.8	9.75 ± 0.8	9.75 ± 0.8
Final length(cm)	10.27±0.95	11.02±0.52	11.27±0.65	10.94±0.61
Length gain(cm)	0.52	1.27	1.52	1.19
Initial weight(gm)	16.25 ± 0.5	16.25 ± 0.5	16.25 ± 0.5	16.25 ± 0.5
Final weight(gm)	17.03±0.59	17.63±0.60	18.23±0.51	17.43±0.62

Weight gain(gm)	0.78	1.38	1.98	1.18
SGR (%)	0.11± 0.34	0.17±0.54	0.26±0.80*	0.15± 0.32

Mean± Standard Error (SE) values *significantly different (p<0.05).

Collection of blood from the fish and separation of serum

Blood from the fish were drawn with the help of a sterilized 2 ml hypodermal syringe and 24 gauge needles directly from the caudal vein containing EDTA as an anticoagulant. Before drawing blood, fishes were anaesthetized with CIFECALM. For serum separation the blood was collected without anticoagulant in serological tubes and stored in a refrigerator overnight. The clot was then spun down at 4000× g for 10 minutes. The serum collected was stored in sterile Eppendorf tubes at -20ºC until used for assays. All the procedures were carried out in the sterilized condition. After drawing blood fishes were given 1% $KMNO_4$ dip treatment and released in to the tank.

Hematological parameter

Total red blood cell (RBC) count

Counting of red blood cells was done following the standard procedure. Blood was drawn in a dry erythrocyte pipette to the 0.5 graduation and then Hayem's solution (Qualigens Fine Chemicals, India) to the 101 mark (dilution 1:200). The pipette was then shaken for 1 minute and counting of the cells was done under X250 magnification. The erythrocytes were counted in 5 group squares (1 group square = 16 small squares) the number of small squares being 80 in 1/400 sq. mm. All cells lying inside the group squares and also the erythrocytes lying to the left and below the demarcation line were counted. Final erythrocyte count was calculated with the formula given below:

$$\text{Total RBC mm}^{-3} = \frac{\text{Erythrocytes in 80 small squares}}{80\times 1/400 \times 1/10\times 1/200}$$

Total white blood cell (WBC) count

White blood cells were counted following the standard procedure. Blood was drawn up to the 0.5 mark and then dilution mixture (WBC diluting fluid, Qualigens Fine Chemicals, Mumbai) was drawn up to 101 marks into the WBC pipette. The filled WBC pipette was gently revolved for 2-3 minute to mix with the diluting fluid; after efficient mixing counting chamber was filled with a small drop of diluting blood. The cells were allowed to settle for 3 minute so counting was done in the large square of the four corners of the chamber & are demarcated by triple line ($1mm^3$). Final white blood cell count was calculated with the formula as follows:

$$\text{Total WBC mm}^{-3} = \frac{\text{No. of leucocytes in 4 square of 1 mm}^2}{4 \times 1/10 \times 1/200} = \text{Total number of cells} \times 50 \text{ cell mm}^{-3}$$

Packed Cell Volume (PCV)

Modified Anderson and Siwicki (1995) [3] method was followed for Packed Cell Volume. In brief, blood was drawn into the graduation mark 100 on the heparinized hematocrit pipette. Both the openings of the pipette were closed with rubber stoppers and centrifuged for 3 minutes. After centrifuging, the capillary tubes were placed on a reading device and the volume was recorded. The hematocrit value was expressed as the percentage fraction of blood cells in the total volume (volume %).

Estimation of Hemoglobin

Estimation of Hemoglobin (Hb) was done by Cyanomethanoglobin method. 0.02 ml of blood was transferred into a tube containing 5.0 ml of Drabkin's reagent. The pipette was rinsed several times with the reagent. The diluted hemoglobin solution was kept for 5 minute to achieve full colour development. The absorbance was measured at 530-550 nm of the unknown sample (A blank) and that of a standard of known hemoglobin content (A standard) against a reagent blank. Final calculation was done with the formula:

$$\text{Hb content (unknown) (g dl}^{-1}) = \frac{\text{A blank} \times \text{Conc. Of the Hb standard (g dl}^{-1})}{\text{A standard}}$$

Test for biochemical parameters

Determination of Blood Glucose

For the determination of Glucose in serum Glucose diagnostic kit (Crest Biosystems, India) was used which was based on Trinder (1969) [28] GOD/POD method.

Determination of Total Protein

Determination of total protein in plasma was done by Biuret method of Gornall *et al.* (1949) [12] based diagnostic kit (Crest Biosystems, India).

Challenge study

Pathogenic strain of Aeromonas hydrophila was received from the Fish Pathology and Microbiology Division, CIFE, Mumbai. A. hydrophila was grown on nutrient broth (HiMedia Ltd, India) for 24 h at 37 C. The culture broth was centrifuged at 3000 g for 10 min. The supernatant was discarded and the pellet was re-suspended in sterile phosphate buffer saline (PBS, pH 7.4) and the OD of the solution was adjusted to 0.5 at 450 nm, which corresponded to 1x107 cells mL-1. After feeding fish with different doses of vitamin C for 45 days, 10 fish from each experimental tank were injected intraperitoneally with 100 µL of bacterial suspension and the mortality was observed for 7 days. Sampling of the survivors was carried out on the 60 d of A. hydrophila infection. The confirmation of the infection was accomplished after re-isolating the bacteria from the dead fish.

Statistical Analysis

All the data were expressed as arithmetic mean ± Standard Error. Statistical analysis of data involved one way analysis of variance (ANOVA) followed by the comparison of means following Least Square Design (LSD) available with SPSS windows 15.0 software. The level of significance were expressed as P- value less or greater than 0.05.

Results

Water quality parameters

The range of water quality parameters *viz.* temperature (27-28.5 ºC), alkalinity (100.00-124.00ppm), dissolved oxygen (5.5-5.8), pH (6.9-7.2), and ammonia- nitrogen (NH_3-N) (0.02-0.06) observed during the study period.

Hematological parameters

Total red blood cell (RBC) count

The total RBC count of the fish fed with vitamin C supplemented diet showed an increasing trend from the 15th to 45th day then decreased considerably on the 60th day (last sampling) in all treatment groups. The group T_2 showed the highest value and were significant ($p<0.05$) in comparison to the control on all the days of sampling (figure 1).

Table 4: RBC count (10^6 mm^{-3}) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0 day	15th day	30th day	45th day	60th day
Control	1.04±0.01	1.05±0.01^{d}	1.07±0.01^{d}	1.11±0.03^{d}	1.05±0.01^{d}
T_1	1.08±0.05	1.17±0.01^{b}	1.30±0.02^{b}	1.48±0.04^{b}	1.20±0.04^{b}
T_2	1.06±0.12	1.23±0.02^{a}	1.37±0.02^{a}	1.85±0.01^{a}	1.32±0.02^{a}
T_3	1.05±0.01	1.13±0.01^{b,c}	1.22±0.02^{c}	1.36±0.03^{c}	1.09±0.02^{c,d}

All data are represented as mean± Standard Error (SE). Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$)

Total white blood cell (WBC) count

The total WBC count of the fish fed with vit C supplemented diet showed an increasing trend from the 15th to 45th day then decreased on the 60th day (last sampling) in all treatment groups. Group T_2 showed the highest value and were significant ($p<0.05$) in comparison to the control on all the days of sampling (figure 2).

Table 5: WBC count (10^6 mm^{-3}) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0day	15th day	30th day	45th day	60th day
Control	17.82±0.03	18.03±0.04^{d}	18.10±0.01^{d}	18.78±0.12^{d}	17.68±0.03^{d}
T_1	17.72±0.03	18.29±0.05^{b,c}	22.27±0.16^{b}	24.26±0.22^{b}	18.77±0.12^{b}
T_2	18.02±0.01	20.83±0.07^{a}	25.08±0.20^{a}	31.19±0.21^{a}	22.17±0.10^{a}
T_3	17.92±0.01	18.23±0.03^{c}	20.44±0.11^{c}	22.66±0.11^{c}	18.05±0.05^{c}

All data are represented as mean± S.E. Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$).

Packed cell volume (PCV)

The PCV level of the fish fed with vit C supplemented diet showed an increasing trend from the 15th to 60th day in all treatment groups. Group T_2 was found to be significant ($p<0.05$) on the 30th day of sampling. The values obtained from all the treatments were insignificant with control (figure 3).

Table 6: PCV (%) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0day	15th day	30th day	45th day	60th day
Control	25.25±0.15	25.34±0.13	26.13±0.21^{a}	27.71±0.35^{a}	33.01±0.22^{a}
T_1	25.39±0.13	25.47±0.11	26.50±0.19 ^{a,c}	27.33±0.16^{a}	31.20±0.33^{b,c}
T_2	25.42±0.13	25.58±0.13	25.79±0.13 ^{a,b}	26.37±0.17^{a}	29.83±1.01^{c}
T_3	25.32±0.11	25.47±0.08	25.57±0.06^{b,c}	27.65±0.12^{a}	31.70±0.10^{a,b}

All data are represented as mean± S.E. Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$).

Total hemoglobin

The hemoglobin content in fish fed with vit C supplemented diet showed an increasing trend from the 15th to 45th day then decreased noticeably on the 60th day in all treatment groups. Group T_2 showed the highest value and were significant ($p<0.05$) in comparison to the control on 30th, 45th and 60th days of sampling. All the treatments were insignificant on the 15th day (figure 4).

Table 7: Total Hemoglobin (gm dl^{-1}) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0day	15th day	30th day	45th day	60th day
Control	5.36±0.04	5.45±0.13	5.60±0.03 ^{d}	5.78±0.02 ^{d}	5.03±0.12 ^{c}
T_1	5.42±0.03	5.46±0.07	6.06±0.08 ^{b}	6.28±0.02 ^{b}	5.29±0.08 ^{a,c}
T_2	5.32±0.03	5.53±0.04	7.02±0.09 ^{a}	7.92±0.04 ^{a}	5.52±0.07 ^{a}
T_3	5.39±0.04	5.39±0.10	5.70±0.13 ^{c,d}	5.88±0.03 ^{c}	5.24±0.04 ^{b,c}

All data are represented as mean± S.E. Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$)

Biochemical parameters

Blood glucose

Blood glucose level in all the groups of fishes fed with vit C supplemented diet showed an increasing trend till the last (60th) days of sampling except T_2 which showed a decrease on day 60, but had significantly ($p<0.05$) higher value from the 15th day onwards as compared to the control(figure 5)

Table 8: Blood glucose level (mg dl $^{-1}$) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0day	15th day	30th day	45th day	60th day
Control	30.88±0.41	25.03±0.28 ^{d}	41.23±0.98 ^{d}	53.25±1.65 ^{d}	62.03±1.76 ^{d}
T_1	31.22±0.20	31.43±1.65 ^{b}	43.84±3.66 ^{b,d}	63.30±1.13 ^{b,c}	64.93±0.40 ^{b,d}
T_2	37.48±0.10	40.49±0.36^{a}	63.35±1.08 ^{a}	91.26±2.87 ^{a}	89.36±2.16 ^{a}

T_3	34.55±0.32	37.88±0.13 ^{c}	41.83±0.70 ^{c,d}	60.63±0.85 ^{c}	61.26±0.74 ^{c,d}

All data are represented as mean± S.E. Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$)

Total protein

The total protein content in fish fed with vit C supplemented diet showed an increasing trend from the 15th to 45th day of sampling in all the treatment groups and then decreased noticeably on the 60th day in the treatment group T_1 and T_2. Group T_2 showed the highest value within the column on day 45th and were significant ($p<0.05$) in comparison to the control on the 15th, 30th, 45th and 60th days of sampling (figure 6).

Table 9: Total protein level (mg dl $^{-1}$) of different experimental groups on various sampling days fed with different doses of vitamin C

Groups	0day	15th day	30th day	45th day	60th day
Control	1.94±0.14	2.13±0.09 ^{c}	2.22±0.06 ^{d}	3.72±0.21^{d}	3.95±0.32 ^{b}
T_1	1.85±0.17	2.70±0.02 ^{a}	3.19±0.06 ^{b,d}	5.54±0.03 ^{b}	2.63±0.04^{d}
T_2	2.04±0.10	3.21±0.29 ^{a}	5.27±0.11 ^{a}	6.57±0.16 ^{a}	5.77±0.10 ^{a}
T_3	1.98±0.09	2.25±0.06 ^{b,c}	3.10±0.37 ^{c,d}	3.72±0.33 ^{c,d}	3.75±0.28 ^{b,c}

All data are represented as mean± S.E. Mean values with different superscript with in a column for a parameter are significantly different ($p<0.05$)

Discussion

In the present study, the cyprinid fish *Labeo rohita* was fed with a feed containing different concentrations of vitamin C ranging from 500 mg kg $^{-1}$ to 1500 mg kg $^{-1}$ feed for 45 days of study period. Data obtained from this study clearly demonstrate that the treatment group T_2 (1000 mg kg $^{-1}$ vitamin C) had the highest significant specific growth rate (table 2) in comparison to the control followed by the group T_1 (500 mg kg^{-1}). It was reported that dietary vitamin C could enhance the growth in channel catfish, *Ictalurus punctatus* (Duncan and Lovell, 1994) [9] and even in Indian major carp, *L. rohita* (Tewary and Patra, 2008; Misra *et al.*, 2007) [27, 20]. Our observation on SGR is in agreement with above observations but disagree with the finding of Sobhana *et al.*, (2002) [26] in Indian major carp, mrigala, *Cirrhinus mrigala*. Previous report showed that the enhancement effects of vitamin C on animal growth might be because of the synthesis of collagen, since vitamin C can promote the transformation of lysine and proline to hydroxylation and hydroxyproline, respectively (Zhou *et al.*, 2003) [30]. While Tewary and Patra (2008) [27] reported that the growth maintaining activity of ascorbic acid had specific effect related to the process of tissue formation, which is required for collagen formation, the concentration of vitamin C in T_2 and T_1 feed might be helpful for proper utilization, because ascorbic acid play an important role in certain aspects of protein metabolism (Shiau and Jans, 1992) [25] and is an essential molecule in the overall health of animals. Hence, from the present investigation it is discernable that a slightly higher dose (500 mg kg $^{-1}$ to 1000 mg kg^{-1}) of dietary vitamin C may be helpful for increasing growth in *L. rohita*.

Haematological parameters are used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions (Agrawal and Mahajan, 1980) [2]. In the present study, RBC and WBC count had increased up to the 45th day in all treatment groups. The increase in RBC count observed in the present study is in agreement with the findings of Duncan and Lovell (1994) [9] in channel catfish, *I. punctatus* and Tewary and Patra (2008) [27] in rohu, *L rohita*, but differs from the finding of Waagbo *et al.*, (1993) [29] in Atlantic salmon (*S. salar*). The increase in WBC count in the present investigation was parallel to that of the RBC count, which supports the observation of Affonso *et al.* (2007) [1] in juvenile matrinxa, *Brycan amazonicum* but differs with the finding of Waagbo *et al.*, (1993) [29] in Atlantic salmon (*S. salar*).

The percentage of packed cell volume (PCV) was found to be enhanced with vitamin C supplemented diet but the values obtained from all the treatments were insignificant against the control except T_2 on the 30th day of sampling which supports the observations of Nayak *et al.* (2007) [21] in rohu, *L rohita.* Other observations on this line come from Dabrowski *et al.* (2004) [7] in Japanese eel with significantly increasing values of PCV. However the present observations are not in agreement with the findings of Lim *et al.* (2002) [17] in channel catfish and Falahatker *et al.* (2006) [10] in sturgeon as they reported decreased PCV values. The total hemoglobin content had shown a significantly increasing trend with vitamin C supplemented group in our study, corroborating with the observations of Falahatkar *et al.* (2006) [10] in great sturgeon (*Huso huso)* and Affonso *et al.* (2007) [1] in juvenile matrinxa, (*B. amazonicum*).

Red blood cell indices (RBC, PCV and Hb) are indicators of fish health as erythrocytes are one of the major production sites of free radicals and some of them trigger peroxidation of saturated fatty acids in their membrane phospholipids, therefore altering their quality (integrity, size) and quantity (Pearce *et al.*, 2003; Kiron *et al.*, 2004) [23, 14] depicts the health status of fish. Menezes *et al.* (2006) [19] reported that WBC are indicators of vitamin efficiency as well as defense mechanism indicators in fish. However, the mechanism by which the high doses of vitamin C influences increase in WBC count as observed in the present study is unclear (Misra *et al.*, 2007) [20], Ren *et al.* (2008) observed that the high PCV values indicate ascorbic acid efficiency and even distribution of iron, without any reduction in the synthesis of hemoglobin that substantiates the findings of the present study.

In the present study, blood glucose levels showed a significantly increasing trend in vitamin C supplemented group. Increased blood glucose level had been reported by Datta and Kaviraj (2003) [8] in catfish, *Clarias gariepinus* upon treatment with vitamin C supplemented diet are in conformity with the present findings. However our findings are at variance from those of Ortuno *et al.* (2003) [22] and Maggioni *et al.* (2004) [18] who reported decrease blood glucose levels in gilthead seabream and *L. vannamei* respectively. Increase in blood glucose level in the present study may be due to the conversion of hepatic glycogen in to glucose, as reported earlier by Datta and Kaviraj (2003) [8] in catfish, *C. gariepinus.*

Total protein level in the present study showed significant increasing values in vitamin C supplemented group which is in conformity with earlier findings of Affonso *et al.* (2007) [1] in juvenile matrinxa but at a variance from other investigation

such as those of Misra *et al.* (2007) [20] in *L. rohita*; where decreased total protein levels have been reported in the vitamin C treated group. Menezes *et al.* (2006) [19] opined that total protein level can be used as an indicator of the nonspecific immune response in fish supplemented with high vitamin C concentration which might have influenced the other nonspecific parameters; this observation is also relevant to the finding of the present study.

However, the present study warrants further studies on most effective dose under pond condition, degree and duration of the resistance offered, administrative regime for different age group of fish and time of application to ensure better growth and healthier harvest. The concentration 500 to 1000 mg kg $^{-1}$ vitamin C was found to be optimum to increase some hemato-biochemical parameter and growth in *L. rohita* under the present experimental conditions.

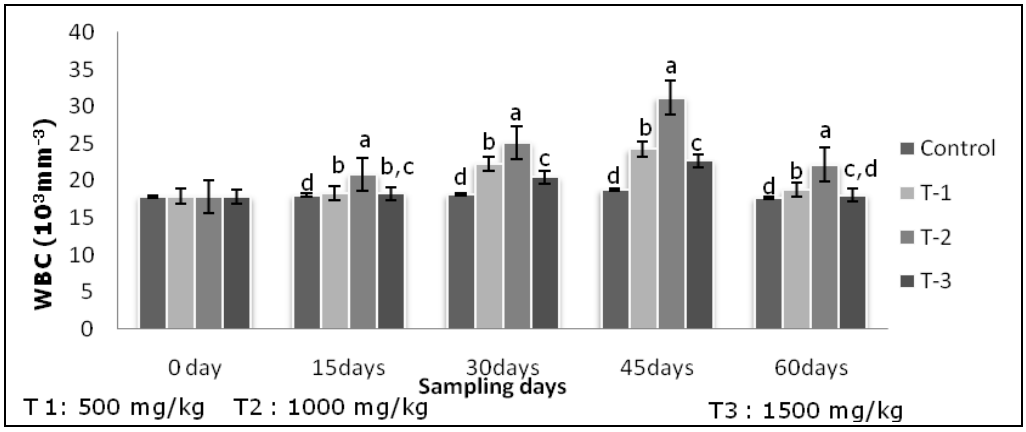

Fig 1: WBC count on various sampling days

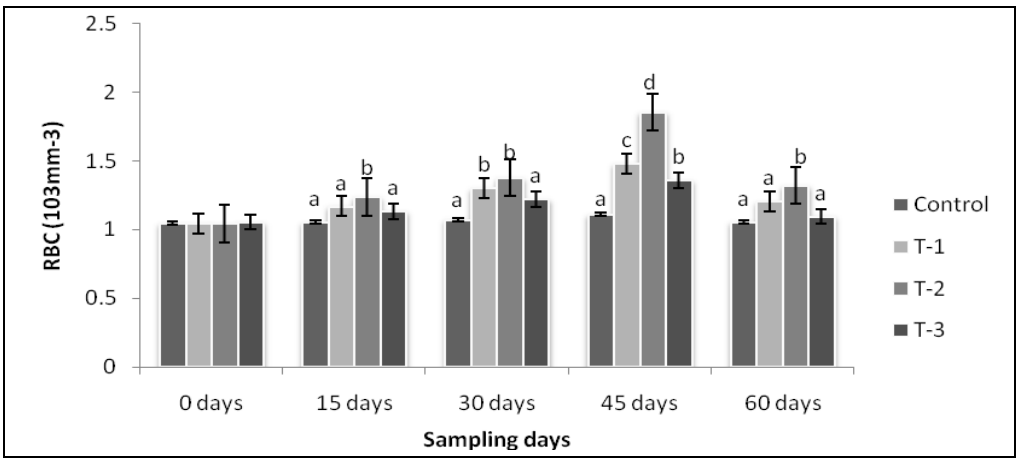

Fig 2: RBC count on various sampling days

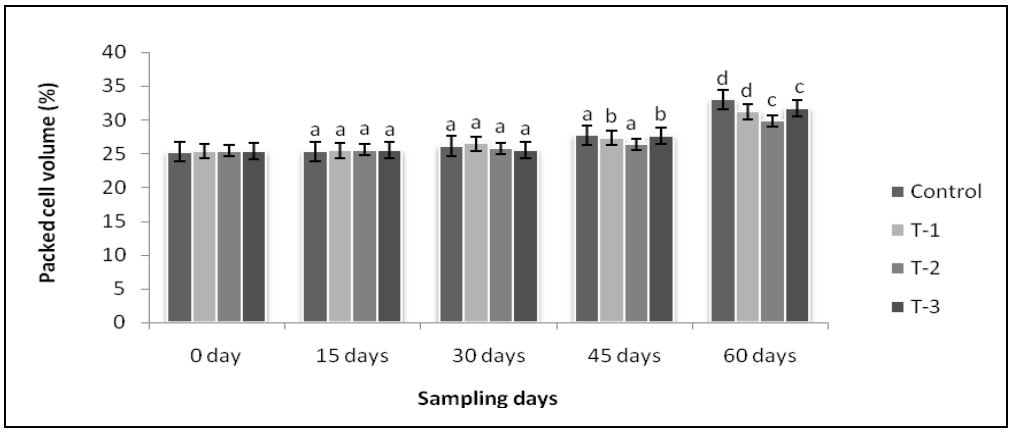

Fig 3: PCV (%) on various sampling days

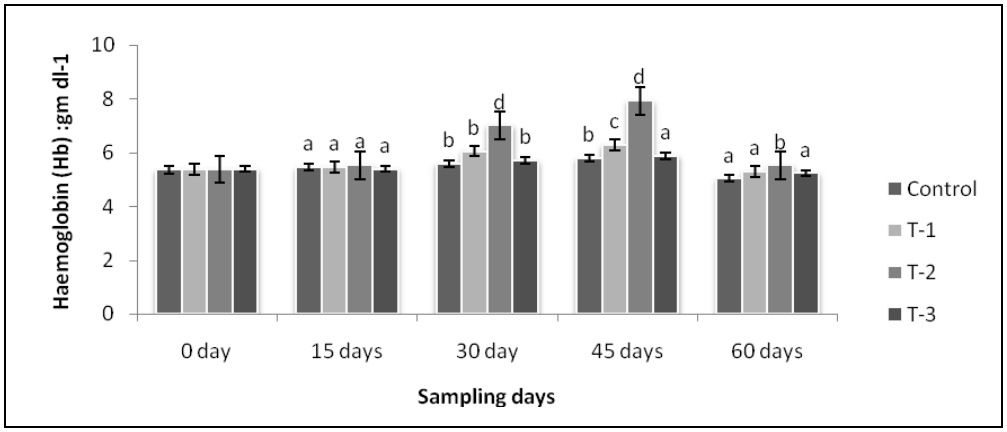

Fig 4: Haemoglobin on various sampling days

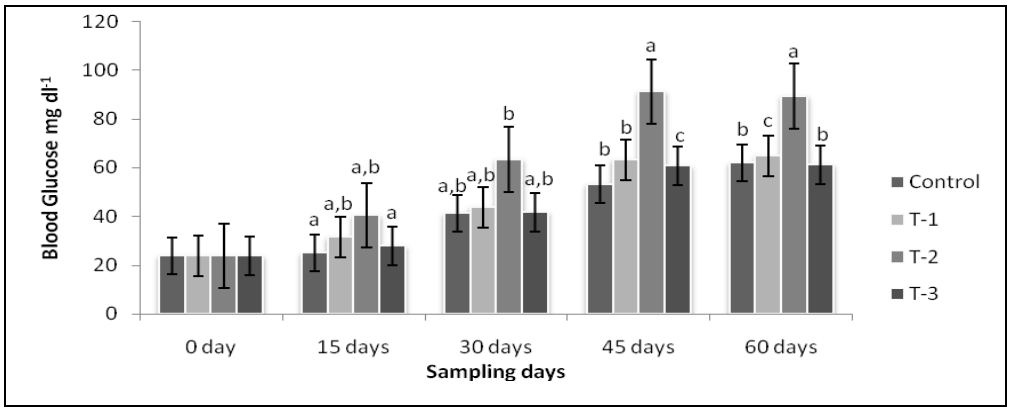

Fig 5: Blood Glucose on various sampling days

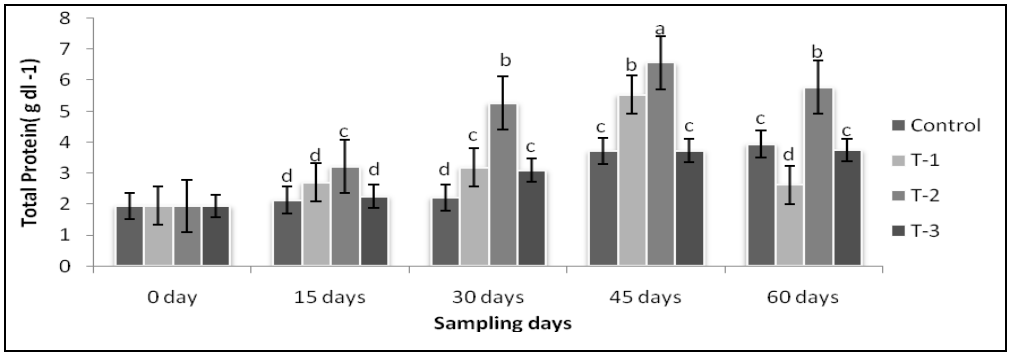

Fig 6: Total Protein on various sampling days

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