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EFFECTS OF FEEDING HIGHER LEVEL OF VITAMIN C L-ASCORBATE-2-TRIPHOSPHATE CALCIUM (LATP) ON GROWTH AND HAEMATO-BIOCHEMICAL INDICES OF COMMON CARP (*CYPRINUS CARPIO* L)

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ABSTRACT

A 42 days feeding trial was conducted to evaluate the effect of feeding higher level of ascorbic acid (AA) on growth response and haemato-biochemical indices of common carp, *Cyprinus carpio*. Four experimental diets supplemented with 0, 1000, 1500 and 2000 mg kg^{-1} AA, designated as control, T1, T2, and T3, were formulated. The ascorbic acid was added as L-ascorbate-2-triphosphate-Ca (LATP) at the expense of wheat flour. Twelve rectangular concrete tanks were randomly stocked with 15 fish (initial average weight of 59 ± 0.56g) each per tank, in triplicate groups. A significantly (p<0.05) higher growth performance indices (FW, WG % & SGR) were recorded in fish fed LATP supplemented diets compared with the controls, while feed conversion ratio showed an opposite trend with the lowest value recorded in T3 fed group. No mortality was recorded through the experiment in all the groups. Fish fed LAT Pbased diets showed significantly higher Hb, RBC, WBC, Hct, total protein, albumin, and A/G ratio, compared with the lowest value recorded in the control. The activities of AST and ALT enzymes in the serum showed statistically higher value in the control group (p<0.05), but decreased with increasing concentration of ascorbic acid in the treatment groups.

Conversely, ALP enzyme activities exhibited the opposite trend. The optimum requirement for maximal tissue storage of LATP in *C. carpio* was found to be 1355 mg kg^{-1}, 1532 mg kg^{-1} and 1216.1 mg kg^{-1} for liver, muscle and kidney, respectively.

KEYWORDS: Ascorbic acid, heamatology, metabolic enzymes, common carp.

1. INTRODUCTION

Ascorbic acid (AA) is a distinct and multi-purpose water-soluble vitamin whose role in several physiological functions such as growth, reproduction, stress mitigation, antioxidation, and immune response cannot be over look (Yamaguchi *et al*., 1995; Gabaudan and Verlhac, 2001). Fish like other animal require AA for normal physiological function, but lack the inherent ability for its biosynthesis. Except in few species, majority of fish generally lack the ability to synthesize vitamin C endogenously due to the absence of L-gulono-1, 4-lactone oxidase, an enzyme responsible for the last step of ascorbic acid biosynthesis (Dabrowski, 1994; Fracalossi*et al*., 2001; Elbaraasi *et al*., 2004). Thus, an exogenous supplementation is required to ensure optimum fish growth and development. Several documented evidence showed that fish fed diets devoid of AA often develop deficiency sign such as spinal deformities (scoliosis and lordosis), reduced growth, internal and fin haemorrhage, lethargy, and increased mortality (Agrawal and Mahajan, 1980; NRC, 2011). Other deficiency symptoms include fin erosion, anorexia, elevated level of plasma triglycerides and cholesterol (John *et al*., 1979).

Ascorbic acid requirement of fish differs with species, size, age, experimental conditions, and nutrient interrelationship (NRC, 2011). The AA requirement for the maintenance of healthy growth and reproduction has been established for several cultured fish species including Channel catfish, Ictaluruspunctatus (51 mg AA kg-1 diet); African catfish, *Clarias gariepinus* (150 mg AA kg-1 diet); *Oreochromis spilurus* (100-200 mg AA kg-1 diet);Common carps, *Cyprinus carpio* (45 mg AA kg^{-1} diet) and *Heterobranchus longifilis* female broodstock (150-200 mg AA kg^{-1}) (EL Naggar and Lovell, 1991; Al-Amoudi et al. 1992; Gouillou-Coustans et al. 1998; Gbadamosi et al. 2008; Adebayo and Fawole, 2012). However, due to the multiple role of ascorbic acid in several metabolic processes, tissue formation and immune responses, increased dietary allowance of up to 8-10 times the requirement for growth has been recommended to reduced mortality and incidence of diseases (Li and Lovell, 1985), and increased reproductive performance in fish (Blom and Dabrowski, 1995). According to the data published by Ishibashi *et al*. (1992), Japanese parrot fish *Oplegnathus*

fasciatus fed elevated level of ascorbic acid showed increased tolerance and reduced mortality when subjected to hypoxic stress compared to the fish fed normal level required for optimum growth.

Therefore, bearing this in mind, the aim of the present study was to investigate the effect of feeding higher level of ascorbic acid L-ascorbate-2-triphosphate-Ca (LATP) on growth performance, haemato-biochemical indices, and health status of common carp*Cyprinus carpio*.

2. MATERIALS AND METHODS

2.1 Diets preparation

Four experimental diets containing 40% crude protein were formulated to contain graded levels of ascorbic acid (Table 1). The ascorbic acid was added as L-ascorbate-2-triphosphate-Ca (LATP, HiMedia Pvt, India) along with other ingredients *viz.* fishmeal, wheat flour and cod liver oil. Ascorbic acid were supplemented to the basal diets at the expense of wheat flours 0, 1000, 1500, and 2000 mg AA kg $^{-1}$ diets, designated as control, T1, T2 and T3, respectively. All the ingredients were grinded, milled and mixed thoroughly to form homogenous blend, then cod liver oil and water were added to form dough. The prepared dough was passed through a feed maker using 2 mm die, and the pellet were air dried. The dried pellet were further chopped into small pieces of required sizes of pellets and then passed through a sieve to obtain homogeneous particle size. Then, the dietswere stored at -20 ^{0}C until use.

2.2 Experimental fish and set-up

The juvenile common carp (*Cyprinus carpio*) used in the present study were obtained from a commercial farm in Jahangirpuri, New Delhi, India, and transported to the department of Zoology, University of Delhi, New Delhi. Fish were acclimatized for one week under aerated condition and fed with the basal diets (without Ascorbic acid). Twelve rectangular concrete tanks (170 L) in outdoor conditions with adequate aeration were used for the experiment. The tanks were randomly stocked with acclimatized fish (initial average weight 59 ± 0.56 g) each per tank, in triplicate following a completely randomized design. The fish were fed with their respective diets to apparent satiation, weighed every two weeks to monitor growth performance and daily ration adjusted accordingly. All groups of fish were fed two times daily at 9:00 h and 18:00 h. The water quality parameters were monitored and maintained at optimal level (temperature 27.9±0.12 - 31.6±0.03^{0}C; pH 7.5±0.07-7.9±0.11; DO 6.6±0.01-

7.5±0.11 mg/l). Two third of water was replenished at weekly intervals till the end of 42 days feeding trial.

Table 1: Preparation of Artificial diets

Ingredients (g)	Control	T1	T2	T3
Fish meal	58.24	58.24	58.24	58.24
Wheat flour	36.76	35.76	35.26	34.76
Cod liver oil	5	5	5	5
Vitamin C	0	1	1.5	2
	100	100	100	100

Table 2: Growth performance and survival rate of *Cyprinus carpio* fed different experimental diets

Treatments	Final weight	Weight gain %	SGR	FCR	Survival (%)
Control	62.80±0.35[a]	33.2626±0. 76[a]	0.6836±0.01[a]	2.52±0. 05[d]	100
T1	67.44±0. 14[b]	43.1006±0. 31[b]	0.8533±0. 005[b]	1.94±0. 01[c]	100
T2	69.15±0. 23[c]	46.7218±0. 50[c]	0.9128±0.008[c]	1.79±0. 01[b]	100
T3	73.53±0. 47[d]	56.0153±1.01[d]	1.0589±0. 01[d]	1.50±0. 02[a]	100
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Table 3: Haematological parameter of *Cyprinus carpio* fed different experimental diets

Treatments	Haemoglobin (g/dl)	RBC (10^6 cells /mm^3)	WBC (10^5 cells /mm^3)	Haematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)
Control	3.95 ± 0.37^{a}	0.32 ± 0.03^{a}	13.36 ± 1.31^{a}	30 ± 0.57^{a}	948.98±111.72	122.15±0.28	13.22±1.50
T1	6.30 ± 0.51^{b}	0.38 ± 0.03^{ab}	28.61 ± 1.54^{b}	40.50 ± 0.86^{ab}	1053.360±74.71	166.99±28.92	15.62±1.61
T2	5.20 ± 0.80^{ab}	0.52 ± 0.07^{b}	53.10 ± 4.77^{c}	46 ± 5.19^{b}	943.83±246.85	108.02±32.43	11.19±0.50
T3	5.25 ± 0.66^{ab}	0.48 ± 0.07^{ab}	65.55 ± 1.19^{d}	43 ± 5.77^{ab}	962.55±271.66	108.50±2.72	13.11±3.39
p-value	**0.138**	**0.133**	**<0.001**	**0.086**	**0.975**	**0.257**	**0.532**

Mean values in the same column with different superscript differ significantly ($p<0.05$). Mean ± SE, n=3

Table 4: Serum biochemical parameters and metabolic enzyme activities of *Cyprinus carpio* fed different experimental diets

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio	SGOT/AST (U/L)	SGPT/ALT (U/L)	ALP (U/L)
Control	2.57 ± 0.9^{a}	0.90 ± 0.01^{a}	1.67 ± 0.10^{a}	0.54 ± 0.04^{a}	575.34 ± 36.12^{b}	145.10 ± 4.21^{d}	68.05 ± 0.77^{a}
T1	4.43 ± 0.16^{b}	2.47 ± 0.08^{b}	1.96 ± 0.24^{ab}	1.31 ± 0.20^{b}	508.70 ± 18.86^{b}	99.85 ± 1.41^{c}	178.40 ± 3.34^{b}
T2	5.79 ± 0.21^{c}	3.92 ± 0.19^{c}	1.87 ± 0.01^{ab}	2.09 ± 0.08^{c}	352.68 ± 8.45^{a}	79.65 ± 2.04^{b}	233.75 ± 3.26^{c}
T3	6.32 ± 0.25^{c}	3.97 ± 0.02^{c}	2.35 ± 0.27^{b}	1.73 ± 0.21^{bc}	338.48 ± 3.99^{a}	26.10 ± 2.07^{a}	279.90 ± 6.69^{d}
p-value	**<0.001**	**<0.001**	**0.157**	**0.001**	**<0.001**	**<0.001**	**<0.001**

Mean values in the same column with different superscript differ significantly ($p<0.05$). Mean ± SE, n=3

2.3 Growth performance and Survival

Fishes were collected from each experimental tank after the completion of feeding trial. Fish were weighed and final weight gains, feed conversion ratio (FCR), specific growth rate (SGR) and survival rate were calculated using the formula below:

Weight gain % = (final weight - initial weight)/ initial weight × 100

Specific growth rate (SGR) = 100 × (LnWt − LnWi)/ t

Feed conversion ratio (FCR) = total feed given (dry weight) (g)/weight gain (wet weight) (g)

Where Wt is final body weight (g), Wi is initial body weight (g); t is experimental duration in days.

Difference in number of fish between the time at stocking and at harvest was determined for estimation of survival. This was expressed in percent (%).

Collection of Blood Sample

Blood samples were collected from six randomly selected fish from each treatment (two per replicate) at the termination of the feeding trial. The fish were anesthetized with tricaine methane sulfonate (MS-222) at 250 ppm in water. Blood was drawn from the caudal vein using heparinized disposable hypodermic needle and transferred into disposable heparinized tube. The collected blood sample was used for haematological study. Blood was drawn from another set of fish (three fish per replicate) for serum collection and frozen in -20 ^{0}C until use.

Hematological Study

Fish hemoglobin (Hb) content was determined by cyanomethaemoglobin method (Larsen and Snieszko, 1964) using Drabkin's reagent. For erythrocyte count,blood sample was diluted 1:200 in Hendricks' fluid and cells were counted on a Neubauerhaemocytometer (Dacie and Lewis, 1984).Total leukocyte count was determined following the method of Shaw (1930).Haematocritwas determined on the basis of sedimentation of blood as described by Akinleye et al. (2011). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the results of Hb, RBC and Hct as described by Dacie and Lewis (1984).

Biochemical Assays

Serum total protein, albumin and globulin

Serum total protein of the fish was determined by Biuret method (Henry et al., 1974), and Albumin by BCG method (Webster, 1977) using a laboratory diagnostic kit (Aspen Laboratories Pvt. Ltd., India). Albumin was subtracted from serum total protein to obtain globulin values, and A/G ratio determined.

FIGURES

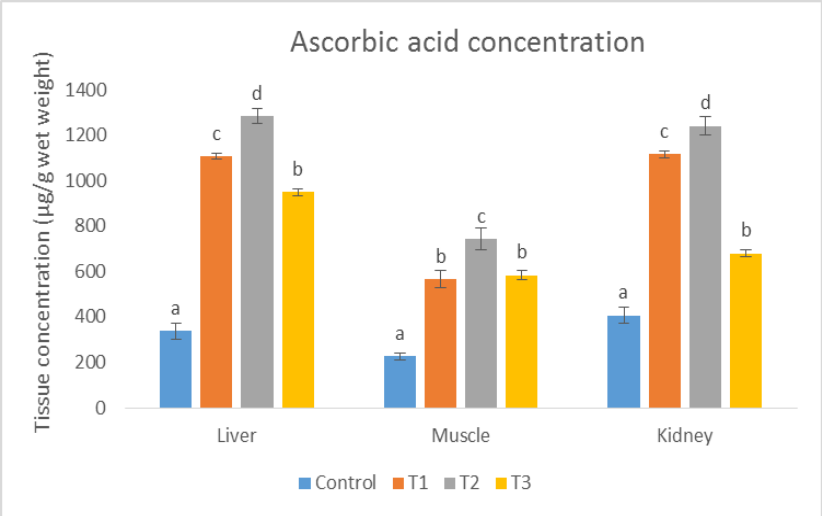

Fig: 1. Ascorbic acid levels in the liver, muscle and kidney of *C. carpio* fed with different experimental diets

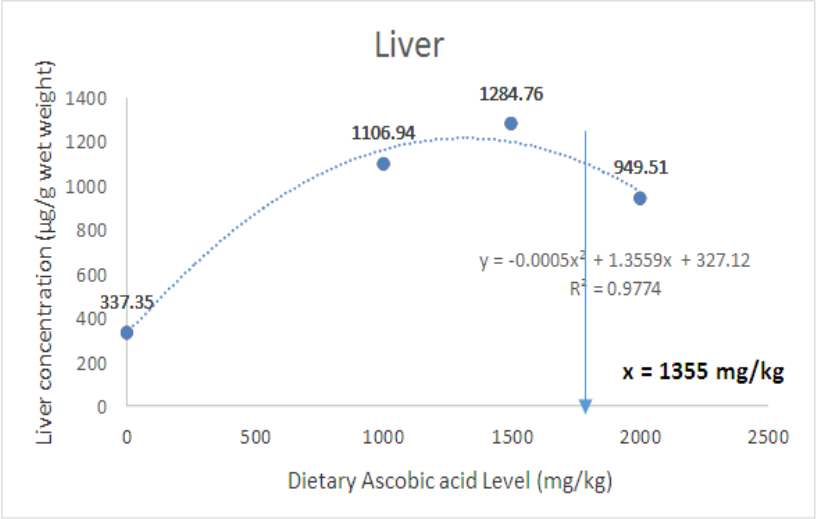

Fig. 2. Second order polynomial of the relationship between dietary ascorbic acid and liver AA concentration of *C. carpio.*

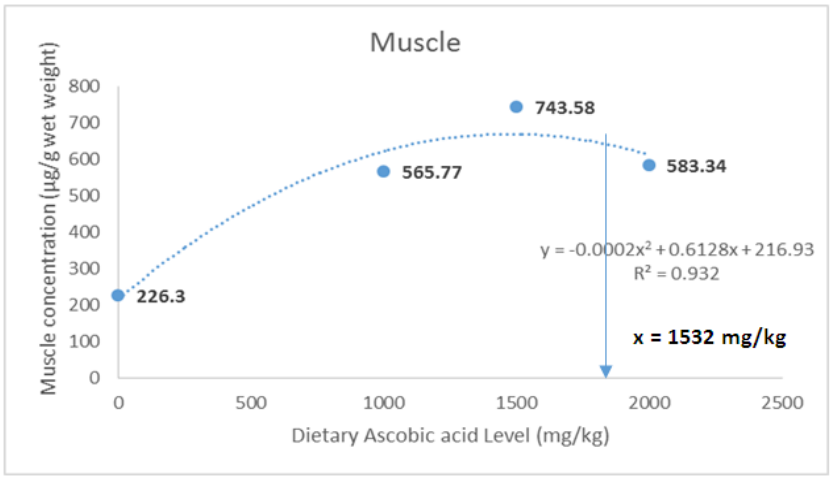

Fig: 3. Second order polynomial of the relationship between dietary ascorbic acid and muscle AA concentration of *C. carpio.*

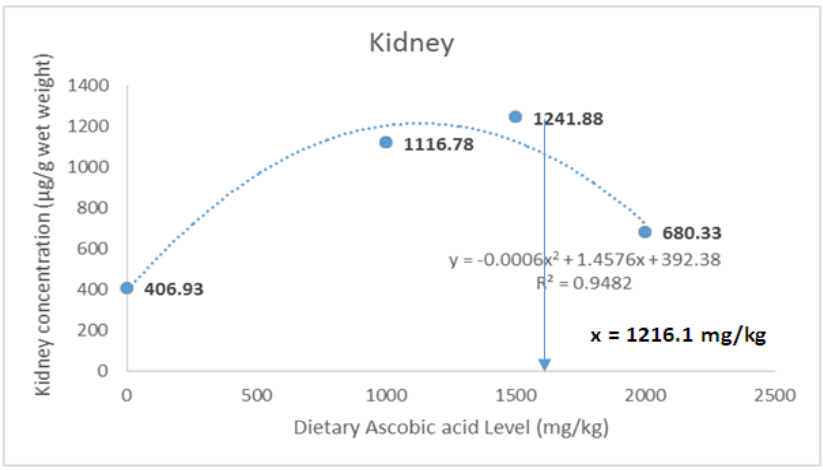

Fig: 4. Second order polynomial of the relationship between dietary ascorbic acid and kidney AA concentration of *C. carpio.*

Metabolic enzyme assay

Serumalanine aminotransferase (ALT/GPT) and Aspartate aminotransferase (AST/GOT) were estimated according to Reitman and Frankel (1957), using a detection kit (Transasia Biomedical Limited, India). Alkaline phosphatase (ALP) was determined by p-Nitrophenyl Phosphate (PNPP) method (Reitman and Frankel, 1957) using kit (Bayer Diagnostics India Ltd., India).

Estimation of Vitamin C from tissue

Two fish from each treatment were collected andanesthetized with tricainemethanesulfonate (MS-222) for 2-3 min. Liver, anterior kidney and body muscles were collected for the assay of vitamin C following the method of Dabrowski and Hinterleitner (1989). Pre-weighed

tissues were homogenized in ice-cold 250 mMperchloric acid ($HClO_4$) containing 5% trichloroacetic acid (TCA) and 0.08% ethylenediaminetetraacetic acid (EDTA). The homogenate was centrifuged at 2700 x g for 20 min at 4^0C. Twenty five microlitre (25 µl) of 0.2% dichlorophenolindophenol (DCIP) was added to 250 µl of deproteinised sample and the mixture was incubated at 37°C for 1 h. Then 25 µl of 1% $KBrO_3$ was added and mixture was incubated at 37°C for 1 h. After 1 h incubation at room temperature, 250 µl of 2% thiourea (in 5% meta-phosphoric acid) was added followed by addition of an equal volume of 2% 2, 4-dinitrophenyl-hydrazine (DNPH) in 12 M H_2SO_4. All samples were incubated for 3 h at 60°C, then 0.5ml of ice-cold 18M H_2SO_4 was added to the samples. The samples were transferred into eppendorf tubes and centrifuged at 11300 x g for 3 min. Absorbance was recorded at 524 nm with a Spectronic 21D spectrophotometer. The blank was prepared in exactly the same manner, except that the buffer was used instead of the sample extract. Standard (20-200µg/ml) was prepared with vitamin C.

Statistical Analysis

All data were analyzed using SPSS 20(SPSS Inc., Chicago, IL, USA). Differences betweendietary treatments were examined by One-way ANOVA (Snedecor and Cochran, 1967) followed by Duncan's Multiple Range tests (DMRT) (Duncan, 1955).Values were presented as means ± standard error.

RESULTS

Growth performance and survival

The growth performance parameters and survival of the fish fed experimental diets is presented in Table 2. Fish fed AA based diets performed better than the control.A significantly higher FW, WG %, and SGR value were recorded in fish fed T3 diet ($p<0.05$). The improvement in growth tends to follows a linear direction as the level of AA increased in the diets. A reverse trend was observed in the case of FCR, with the lowest value recorded in T3 fed group compared to the higher value in the control. No mortality was recorded in all the groups throughout the experimental period.

Hematological Study

Haemoglobin, RBC, Hctcontent, MCV, MCH and MCHC values are given in Table 3. The dietary ascorbic acid supplementation hada significant effect on the Hb, RBC, and Hct content, with the highest value recorded in T1 and T2 groups, respectively. Although, statistically similar ($p>0.05$) value were observedbetween the other groups and control, but

there values were found to be higher.A significantly higher ($p<0.05$) WBC count was recorded in fish fed 2000 mg/kg AA (T3) compared with the control.The MCV, MCH and MCHC value did not differ ($p>0.05$) among the experimental groups.

Serum biochemical parameters

Total protein, albumin, globulin and A/G ratio of different experimental groups are given in Table 4. Significantly higher total protein and albumin values were recorded in T3 fed group, which was not differ statistically ($p>0.05$) from fish fed T2 diet. The globulin value showed no significant variation among the experimental groups. A/G ratio was highest in T2 group, whereas the control group recorded a significantly ($p<0.05$) lowest value compared with the AA fed groups.

Metabolic enzyme activities

Transaminases (AST & ALT) and alkaline phosphatase (ALP) enzyme activities value are shown in Table 4. A statistically higher AST and ALT enzymes activities were observed in the control group and decrease with increasing concentration of ascorbic acid in the treatment groups, whereas ALP enzyme activities exhibited the opposite trend.

Ascorbic acid concentrations in tissue

The ascorbic acid concentration in liver, muscle and kidney of the experimental fish increased significantly ($p<0.05$)with increasing level of AA in the diets. However, at higher supplementation above 1500 mgAA/kg, a decline in the tissue concentration of ascorbic acid was observed(Fig. 1). The dietary level of AA showed a second order polynomial relationship with tissue concentration in the liver ($y = -0.0005x^2 + 1.3559x + 327.12$, $R^2 = 0.9774$), muscle ($y = -0.0002x^2 + 0.6128x + 216.93$, $R^2 = 0.932$), and kidney ($y = -0.0006x^2 + 1.4576x + 392.38$, $R^2 = 0.9482$) (Fig, 2, 3, & 4, respectively).

DISCUSSION

The inability of many fish species to synthesize AA endogenously has led to an array of research findings in other to establish the requirement for optimum growth. The quantitative requirement for several fish species studied show far ranges between 25-200 mg AA kg^{-1} diets, depending on the age, size, species, stages of development, and culture conditions (Stickney et al. 1984; EL Naggar and Lovell, 1991; Al-Amoudi et al. 1992; Adebayo and Fawole, 2012). However, due to the multiple role of ascorbic acid in several physio-metabolic processes, tissue formation and immune responses, increased dietary allowance of

up to 8-10 times the requirement for growth has been recommended to reduced mortality and incidence of diseases (Li and Lovell, 1985). In the present study we supplemented the diets of common carp with AA 10 times more than the requirement for growth, and observed a significant improvement in growth and nutrient utilization indices. The group of fish fed 2000 mg AA kg^{-1} performed better in percent weight gain, SGR and FCR compared to the other fed groups. Increase in growth has been reportedto be a function of the nutritional quality of a diets, and often considered as a positive indices in nutritional requirement studies (Stickney2000). Faramarzi (2012) reported that *Cyprinus carpio* fed diets containing high level (800-2000mg AA kg^{-1}) of vitamin C performed better in terms of growth and nutritional indices compared tothe fish fed less or no ascorbic acid. Similarly Tewary and Patra (2008) stated that *L. rohita* fed ascorbic acid supplemented diet showed higher SGR, PER and lower FCR value up to 1000 mg AA/kg compared with the control group. Hence, the increased growth observed in the present study can be attributed to the role AA in the process of tissue growth and repair and nutrient utilization, since ascorbic acid plays an essential physiological function in some aspect of protein metabolism and collagen formation (Shiau and Jan 1992; Tewary and Patra 2008).

Haematological indicesare often employed to evaluate the physiological and health status of fish in response to different diets or environmental variables (Jahanbakhshi *et al*., 2013), and any reduction in these parameters are often related to their health conditions. The present study showed that AA supplementation had a positive influence on the Hb, RBC, WBC and hematocrit value of the treatment fish compared to the control. The reason for the reduction in the value of Hb, RBC and Hctin the control may be attributed to the nutritional deficiency often caused by the reduction in the absorption of iron, a very important mineral for blood formation. It is a well-documented factthat ascorbic acid deficiency could result in anemia in fish due its role in the absorption of iron and consequently in the synthesis of haemoglobin (Shiau and Jan, 1992, Hsu and Shiau, 1999). Nsonga et al. (2009) reported that *Oreochromis karongae* fed diets supplemented with AA performed better compared to the control fish in terms of hematological indices. Similar report weremade by Zhou et al. (2012) in juvenile cobia *Rachycentron canadum* fed vitamin C supplemented diets. Hence, the lower RBC and Hb values observed in the control group could be said to be a sign of anaemia, which may not be unconnected with the vitamin C deficiency as seen in most studies (NRC, 2011). White blood cell plays a significant role in the immune function of fish, and elevated level of WBC observed in the group fed 2000 mg AA / kg diets indicated that adequately high level of AA

could confer immuno-competency on *C. carpio* before any incidence of diseases and or stress condition. Gbadamosi et al. (2008) reported that ascorbic acid supplementation elevated the level of WBC in *C. gariepinus* fed AA supplemented diets, thus enhance the immune response. Similar observation was made by Tewary and Patra (2008) in *L. rohita* fed AA up to 1000 mg/kg, but a decreased WBC value was observed when the inclusion level increased to1500 mg/kg. The reason for the variation compared to the present study might be due to the form of ascorbic acid used (L-ascorbate-2-triphosphate-Ca) and species differences in the utilization of different forms of ascorbic acid.

Serum total protein content plays a prominent role in determining the nutritional status and welfare of an animal including fish. This circulating mobile proteins can further be grouped into albumin and globulin, whose role in innate defense mechanism of fish has been reported(Kumar et al. 2007; Fawole et al. 2015). In the present study, total protein and albumin content were found to increase with increasing addition of AA, which is similar to the report of Huang et al 2014) in GIFT tilapia. Except with fish fed 2000 mg/kg AA, no differences were found in the globulin content of fish fed T1, T2 and control diet, wherein a significant increase was observed in the A/G ratio value. The higher A/G ratio recorded in this study is at variance to those reported by Tewary and Patra (2008) who recorded higher A/G ratio in control (without AA). Although, globulin data was not presented but we are of the opinion that the reason for the disparity may be due to low concentration of globulin in their AA supplemented groups compared with the control. Nevertheless, in the current study, the significant reduction in total protein, albumin and A/G ratio observed in the control group points in the direction of nutritional deficiencies and or depression of the immune function, since decreased albumin level are often associated with dietary deficiency andused as index of liver damage(Silverman et al., 1986). It should also be noted that protein plays a major role in the process of tissue repair, thus making its concentration low in the serum as seen in the control fed group (Neff, 1985).

The presence of some metabolic enzymes such as AST and ALT in the serum or plasma are often indicative of hepaticdamage (Racicot et al., 1975). The lower AST and ALT activities recorded in the group fed mega dose (1000-200 mg/kg) of AA might be due to the role of ascorbic acid in the protection of tissues from oxidative damage (NRC 2011). This damage usually occur when ROS is over produced, thereby inflicting damages on vital organs. However, ascorbic acid is a potent water soluble antioxidant that help in counteracting the

effects of this ROS, which may be the reason for the lower activities of these enzyme in the serum of the fish fed AA supplemented diets. According to Chen (2003) and Zhou et al. (2012), feeding fish with diets low or devoid of vitamin C may result in reduced antioxidant capacity, and more susceptibility to inflammation as a result of tissue damage. Higher serum ALP activity was recorded in the groups fed AA based diets, and this may be linked to the role of AA in stimulating alkaline phosphatase activity, a marker for osteoblast function (Morton et al., 2001). Also, since ALP is a hydrolase enzyme that catalyse the breakdown of phosphate-containing compounds in the body (Zhou et al. 2012), so increased activity of this enzyme in the present study gave an insight into the utilization of L-ascorbate-2-triphosphate-Ca by the fish, and its positive effect onthe growth performance. The result of the present findings is in accord with the previous study in juvenile cobia *Rachycentron canadum* and GIFT tilapia *Oreochromis niloticus* fed AA supplemented diets (Zhou et al. 2012; Huang et al. 2014).

Tissue concentration of AA is directly related to its dietary intake, and, liver and kidney are an important storage organs for ascorbic acid in fish (Verlhac et al. 2015). In the present study, increased dietary level of ascorbic acid resulted in a significantly elevated levels of AA in the tissue. However, a sharp decline in AA was observed above 1500 mg/kg supplementation level in all the tissue, thus indicating that the organs are saturated with ascorbic acid. This is in conformity with the previous report that excessive dietary AA cannot accumulate in the tissue once saturated (Zhou et al. 2012), and requirements for tissue saturation are much higher than that for normal fish growth for the prevention of deficiency symptoms (Lim and Lovell 1978).Similar reported were given in *Lateolabrax japonicus* (Ai et al. 2004); *Epinephelus malabaricus* (Lin and Shiau 2005);*Oreochromis karongae* (Nsonga et al. 2009); and *Rachycentron canadum* (Zhou et al. 2012). Nevertheless, due to a decline in AA concentration above 1500 mg/kg, a second order polynomial was used to determine the exact maximal tissue level. It was observed that the maximal storage level for C. carpio were found to be 1355 mg/kg, 1532 mg/kg and 1216.1 mg/kg for liver, muscle and kidney, respectively. Theselevels will ensure adequate protection of the organ against oxidative insult and help in the regeneration of vitamin E after oxidation. Several authors have also reported that the level of vitamin C required for maximum tissue storage is always higher than the requirement for maximum growth (Gouillou-Coustans et al. 1998; Ai et al. 2004; Huang et al. 2014).

In summary, the supplementation of ascorbic acid at higher dose showed a positive effectson the growth and nutrient utilization indices in *C. carpio*. The haemato-biochemical parameters and tissue concentration of AA were also significantly influence due to the feeding of high level of ascorbic acid. Therefore, it is suggested that inclusion of higher dose of AA should not be more than 1500 mg/kg in the diets of common carp formaximal tissue storage, and to confer immuno-competency onthe fish before any incidence of diseases and or stress condition.

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