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Immunostimulatory Effect of Supplementary Diet Vitamin C on Growth, Haematology, Survival and Immunity of Fish, *Catla Catla.*

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Abstract: The aim of this study was to assess the effects of vitamin C (Vit C) on the growth, blood parameters, haematocrit and survival in juvenile of carp fish Catla catla. Vitamin C (Ascorbic Acid) requirement of juvenile Catla catla was also studied by incorporating varying levels of Ascorbic acid in a 20% crude protein diet to obtain 0, 10, 20, 40, 80 and 120 mg ascorbic acid equivalent kg^{-1}diet. Juvenile fish of 5.25g ± 0.03g initial body weight were used for the present study. After 120 days of the experiment, the fish fed AA supplemented diets induced significantly (P<0.05) higher specific growth rate (SGR), protein conversion efficiency (PCE) and protein efficiency ratio (PER) and significantly (P<0.05) better feed conversion ratios (FCR) than fish fed with non supplemented diet. Fish fed non supplemented diet recorded a 15% rate of mortality, where as those fed with a diet supplemented with 120 mg ascorbic acid kg^{-1} had mortality as low as 0.05%. Haematological indices showed a significant increase (P<0.05) with dietary AA levels. The maximum growth rate was observed in fish fed with 120mg kg^{-1} of ascorbic acid, whereas the stunted and uneven growth appeared at lower levels of ascorbic acid. However 80 mg ascorbic acid kg^{-1} supplemented diet is considered as the optimal level required for juvenile Catla catla. The results presented in the current study revealed that vitamin C is a beneficial dietary supplement for improving the growth performance due to more supply of oxygen to deeper tissue of carp fish, Catla catla. A satisfactory level appeared to be 120mg ascorbic acid (AA) kg diet which is therefore the level recommended for supplementing common carp food in aquaculture for high yielding.

Keywords: Catla catla, ascorbic acid, supplemented diet, specific growth rate, Feed Conversion Ratio, Supplementation.

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I. INTRODUCTION

The aquaculture industry is growing rapidly worldwide, but continues to contend with some issues such as disease, product quality, feed contamination and environmental impacts. Fish nutrition and its impact on animal welfare is an important aquaculture issue. Indigent perception of the fish dietary requirements in formulation and development of formulated artificial diets for intensive aquaculture have resulted in mortality rates. Vitamins are predominant requisite nutrients for aquatic animals. Several morphological and functional abnormalities have been reported in various fish species impoverished of vitamins[1].

It has proven that dietary levels of vitamins C enhance the antibody production and immune memory in juvenile of milk fish[2]. Additionally, some previous research findings indicate that dietary supplementation with immune - modulatory vitamins such as vitamins can improve the immune response and disease resistance of a variety of cultured fish species[3], while some other studies have failed to show pragmatic responses with over escarpment of such vitamins[4, 5]. So, it is clear that the biological role played by vitamins C is very indispensable for the sustained growth and health of many living organisms as well as fish.

Vitamin C (Ascorbic Acid, AA) is an essential nutrient in aqua feeds, and is an indispensable nutrient required to maintain physiological processes such as normal growth, immunity and reproduction of different animals including fishes[6]. AA is water soluble and is essential for several metabolic functions including the antioxidant system. Most fish, including carp fish *Catla catla,* are not capable of vitamin C biosynthesis[7], due to absence of the enzyme L-gulonolactone oxidase, which is responsible for synthesis of AA[8].

Vitamin C is involved in the maintenance of body physical and metabolic functions. However, there is a great deal of studies that show discrepancy results about its effects. From the time it was first isolated in 1928, multitudinous studies have been done on its biochemical and pharmacokinetic properties, its functions and even the role of this molecule in neurophysiology. It is important to diagnosticate the role vitamin C has in the maintenance of oxide and reduction (redox) balance, as well as the possible effect it may have on the treatment of chronic degenerative diseases, autoimmune diseases and cancer[9].

The requirement of AA is varying among fish species, but intraspecies differences such as fish strain, size and age also affect the dietary requirement. The amount of AA that must be added to the diet for normal function is also dependent on the form of the vitamin C that is added to the diet[10].

Vitamin C has been shown to stimulate immune response in fish[11, 12, 13]. Vitamin C has been used in fish foods for ameliorating fish immunity and growth [14]. Fish cannot synthesize vitamin C, because they don't synthesize themselves the L-glutonolactone oxidase enzyme, which is obligatory to convert L-gutonic acid to ascorbic acid[15]. For this reason, it is necessary to summate vitamin C in fish feed.

Ascorbic acid plays a role in oxidant defence and therefore participates in the protection against certain contaminants. Important contaminant-induced eccentricities in fish such as skeletal deformities, fin erosions and skin lesions are being related to dietary ascorbic acid delinquency and/or over utilisation of ascorbic acid store in the defence mechanism against toxicants[16, 17].

Vitamin C is an indispensable and multifunctional micronutrient. It plays important roles in improving immune function[18], improving growth[19], providing good health, feed conversion, survival [20], resisting stress[21] and oxidation[22]. Most fish species are not capable of vitamin C biosynthesis[23], due to the absence of the enzyme L-gulonolactone oxidase necessary for ascorbic acid synthesis[24]. Vitamin C also plays an important role in animal health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stress[25]. It is suggested that antioxidant function of these micronutrients could magnify immunity by preserving the functional and structural integrity of immune cells. In this respect, the need for specific nutrients may be increased during infection which could require the feeding on diets formulated for optimal immune competence rather than growth and survival.

Although most vertebrate animals synthesize L - Ascorbic acid, several species such as primates, guinea pigs, fish, bats, insects and some birds lack the ability to produce and are thus dependent upon a dietary source of vitamin C. Fishes are unique among these animals in that they have a system to store a chemically stable form of vitamin C which appears to allow metabolism of this compound differently from other vitamin C requiring organisms[26]. Vitamin C is capable of maintaining sulphydral compounds in a reduced state particularly in severe redox reactions[27].

Vitamin C deficiency has also been shown to produce various abnormal signs in various fish including slow growth rate[28], impaired wound healing[29], increased susceptibility to bacterial diseases [30], lower survival rates[31]. However, vitamin E is among the most important a nutrient influencing the fish immune system and its supply can reduce mortality and improve fish performance[32, 33].

The objective of the present study was to assess the effects of different levels of dietary vitamin C on growth, haematological and immunological parameters of *Catla catla* fingerlings.

II. MATERIALS AND METHODS

Experimental set up: Juvenile *Catla catla* (4.25g ± 0.04g) were collected from Fish Breeding Centre, Kalyanidam, near Sri Venkateswara University campus. To eliminate possible external parasites, fish were treated in sodium chloride bath of 2 mg L^{-1} every two days during two weeks of acclimatization. During acclimatization the fish were offered a maintenance diet containing 30% protein, twice a day. The experimental tanks were filled to the brim with well water. Continuous aeration was provided with aerators that maintained oxygen levels above 5.5 mg L^{-1} (Table-1).

Table 1: Composition of the diet used through out of the experimental period (dry weight).

Ascorbic acid Content	Various Vitamin C levels in Experimental Diet (g kg-1 dry weight)						
	Control 0mg	**10mg**	**20mg**	**30mg**	**40mg**	**80mg**	**120mg**
Ingredients							
Fish meal	630	630	630	630	630	630	630
Meat powder	65	65	64	65	66	65	65
Wheat flour	100	99	100	102	100	102	100
Soybean cake	55	54	55	56	57	54	56
Fish oil	45	45	44	47	46	45	47
Soybean oil	45	47	46	45	44	47	48
Lecithin	35	35	33	34	36	37	35
Vitamin mixture (Vitamin E free)	15	16	15	14	17	15	18

Mineral mixture	15	14	16	15	17	16	15
Salt	5	5	6	4	5	6	6
Proximate Composition (% Mean ± SD)							
Crude protein	30.18±1.5	31.19±1.2	30.17±1.5	31.15±1.6	31.5± 1.3	30.16±1.2	30.17±1.5
Crude lipid	15.12±1.6	15.14±1.2	15.15±1.7	16.15±1.3	15.13±1.2	15.17±1.8	15.14±1.4
Moisture	15.25±1.4	15.26±1.1	15.25±0.8	15.24±1.5	15.28±1.5	15.29±1.9	15.27±1.6
Ash	24.70±1.6	24.69±1.3	24.65±0.9	24.66±1.8	24.72±1.4	24.73±0.9	24.75±1.8
Crude Energy (kcal kg-1)	3215±1.5	3216±0.8	3215±1.04	3214±1.4	3216±1.2	3215±1.4	3216±1.4

Experimental design: Twenty (20) tanks of 250L were stocked with 40 fish in each. Fish were acclimatized for one week and fed a basal diet containing 30% crude protein. Five experimental diets with 5 varying levels of L-ascorbic acid (0 mg, 10 mg, 20 mg, 40 mg, 80mg and 120 mg kg^{-1} diet) with 99.9% purity, containing 40% protein level were then assigned in triplicate to the experimental unit in a completely randomized design. The fish were fed to adequately twice daily at 10% of body weight in pellet form.

Sample Collection: The growth parameters of the *Catla catla* fingerlings were assessed by taking their body weight at an interval of 15 days as in fish body weight gain (g) and length (cm) measured individually to make adjustment to feeding of fish. Feed intake was taken into account for calculations of feed conversion ratio (FCR), protein conversion efficiency (PCE) and protein efficiency ratio (PER). Prior to sampling, fish are maintained without feeding for 24 hours. Every two weeks fish were massacarized for blood, liver and muscle tissues for analysis. The growth performance was assessed using the following standard formulae for different growth parameters.

Growth Parameters: The growth parameters of the *Catla catla* fingerlings were assessed by taking their body weight at an interval of 15 days. The animals were kept starved for 8 h before body weight measurement. The growth performance was assessed using the following standard formulae for different growth parameters.

Weight gain % = (final weight – initial weight) / (initial weight) x 100.

Specific growth rate (SGR) =
100 (loge average final weight −loge average initial weight) /number of culture days.

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

Feed efficiency ratio (FER) = wet weight gain (g)/ total dry feed intake (g).

Protein efficiency ratio (PER) = total wet weight gain (g) /crude protein fed (g).

Apparent net protein utilization = (Final carcass protein- initial carcass protein) / protein fed X 100.

Survival (%) = (total number of animals harvested/total number of animals stocked) X 100.

Haematological parameters analysis: At the end of the experiment, blood samples were taken from the caudal vein of non-anaesthetized fish by sterile syringe rinsed with EDTA as an anticoagulant. Fishes were anaesthetized in 100 ppm clove powder solution, and then blood samples were collected via venipuncture and inspirited into a microcentrifuge tube. The first sample was transferred to an eppendorf tube coated with heparin as an anticoagulant and was used for haematological indices determination including hematocrit (Ht), total red blood cell count (RBC) and total leukocyte count (WBC).

The collected blood was used for determination of erythrocyte count[34], haemoglobin content[35] and haematocrit value[36].

Observations for clinical signs on fish continued throughout the experimental period. Water in control and experimental tanks was changed at least $3/4^{th}$ for every 48 hours and complete cleaned up of fish tanks was done every two weeks. Water quality was monitored using with standard methods of APHA[37].

Water quality monitoring: Water quality parameters remained in acceptable ranges during the experimental period and showed no significant differences (P>0.05) across treatment diets. Temperature ranged between 22.4^{o}C ± 0.05 to 24.3^{o}C ± 0.02. Dissolved oxygen concentration averaged 6.8 mg L^{-1}. The pH values ranged from 7.34 ± 0.05 in treatment 0mg ascorbic acid kg^{-1} to 7.47 ± 0.05 in treatment 120mg ascorbic acid kg^{-1} diet. Ammonia averaged 0.12 ± 0.2 mg L^{-1} and means were not significantly different (P>0.05).

Statistical Analyses

All data were subjected to a one-way analysis of variance (ANOVA) after confirmation of normality and homogeneity of variance. Significance of the differences between means was tested using Duncan's multiple range test (Duncan, 1955) (p<0.05) and using T-test to compare the means of treated groups against that of the corresponding control. All assays were carried out in triplicates and data are shown as mean ± SD for each dietary group.

III. RESULTS

Fish, juveniles of *Catla catla* fed diets without ascorbic acid had significantly lower (P<0.05) weight gains than fish fed with ascorbic acid supplemented diets. Fish without ascorbic acid supplementation stopped growing at week 8 and began to lose weight. Fish fed ascorbic acid - supplemented diets did not stop growing during the 15-week feeding period and reached a significantly higher (P<0.05) weight than fish without dietary ascorbic acid. After 8 weeks of supplementation, 120 mg ascorbic acid kg^{-1} diet gave mean weight of 29.59g**,** while 80 mg ascorbic acid kg^{-1} diet gave mean weight of 26.66g. The 40mg ascorbic acid kg^{-1} diet promotes 21.65g weight increase for juveniles *Catla catla* (Table 2).

Table 2: Final body weight (FBW), weight gain (WG) (g/fish), total length (TL), feed conversion ratio (FCR), specific growth rate (SGR), condition factor (CF), Food Conversion Ratio (FCR), Protein Convertion Effeciency (PCE) and Protein Efficiency Ratio (PER) of *Catla catla* fed the experimental diets for 8 weeks.

Diet Supplemented AA (mg K^{-1})	Initial Mean Weight(g)	Final Mean Weight (g)	Weight gain (g/fish)	SGR (%day^{-1})	Total Length (cm)	Condition factor	Survival Rate (%)
0mg	5.12 ± 0.02	8.6 ± 1.3	3.48 ± 0.6	2.06 ± 0.25	4.5 ± 0.04	1.04 ± 0.31	100
10mg	5.13 ± 0.02	10.8 ± 1.1	5.67 ± 1.4	2.12 ± 0.33	5.4 ± 0.03	1.04 ± 0.05	100
20mg	5.14 ± 0.01	15.1 ± 3.5	9.96 ± 1.6	2.04 ± 0.15	6.3 ± 0.01	0.96 ± 0.02	100
30mg	5.12 ± 0.06	18.2 ± 7.5	13.08 ± 1.7	2.15± 0.32	6.9 ± 0.02	1.04 ± 0.09	100
40mg	5.15 ± 0.02	21.65 ± 4.9	16.40 ± 1.4	2.25 ± 0.25	7.5 ± 0.04	1.03 ± 0.11	100
80mg	5.18 ± 0.05	26.66 ± 8.9	21.48 ± 1.6	2.99 ± 0.49	7.9 ± 0.04	1.06 ± 0.16	100
120mg	5.19±0.15	29.59± 2.8	24.40±1.8	3.25 ± 0.25	8.6 ± 0.08	1.06 ± 0.14	100

Values are Mean ± SD of six individual observations. Mean values are significant at p < 0.05.

After 8 weeks, the specific growth rate, feed conversion ratio, protein conversion ratio and protein efficiency ratio are significantly lowered (P<0.05) in fish without ascorbic acid supplementation than those fed with ascorbic acid supplemented diets (Table 2). In the present study maximum FCR (21.25), PCE (39.55) and PER (0.35) are observed at the dose of 120mg at the end of 8weeks exposure periods.

Effect of vitamin C on haematology, ascorbate levels in liver and muscle tissues are represented in Tables-3, 4 and 5. Haematological values are varied and increased significantly (P<0.05) with increase of dietary ascorbic acid level. Diet without ascorbic acid supplementation showed the lowest values, while diet 120mg ascorbic acid kg^{-1} indicated the highest haematological activities (Table 3). The red blood cell counts, haemoglobin and packed cell volume showed a significant increase (P<0.05) with increase in values of ascorbic acid whereas WBC showed a decrease with increase of concentration of vitamin C and exposure periods (Table-4). The ascorbate levels in both liver and muscle tissues are gradually increased in all treatments with increase of concentration of Ascorbic acid, except in the 0 mg kg^{-1} treatment (Table 5).

Table 3: Plasma Proteins, Food Conversion Ratio (FCR), Protein Convertion Effeciency (PCE) and Protein Efficiency Ratio (PER) of *Catla catla* fed the experimental diets for 8 weeks.

Diet Supplemented AA (mg K^{-1})	Plasma Proteins (g/dl)		FCR	PCE	PER
	Initial	Final			
0mg	1.56±0.14	1.55±0.15	12.5± 1.08	2.32 ± 1.07	0.06±0.04
10mg	1.55±0.26	2.75±1.12	14.4 ± 1.17	12.28±1.06	0.08±0.05
20mg	1.52±0.22	2.78±1.13	15.6 ± 1.10	22.26±1.09	0.18±0.05
30mg	1.54±0.12	2.85±1.26	16.9 ± 0.77	26.35±1.05	0.22±0.18
40mg	1.53±0.15	3.25±1.15	18.6 ± 0.17	32.61±2.08	0.26±0.19
80mg	1.52±0.14	3.56±1.16	19.4 ± 1.53	36.25±2.05	0.32±0.16
120mg	1.53±0.13	3.89±1.14	21.25±0.25	39.55±2.06	0.35±0.13

Values are Mean ± SD of six individual observations. Mean values are significant at $p < 0.05$.

Table 4: Haematological parameters of *Catla catla* fingerlings fed 8 weeks with diets containing different levels of Vitamin C.

Diet Supple-mented AA (mg K^{-1})	RBC (×106)	WBC (×103)	PCV (%)	Hb (gr/dl)	MCV (fl)	MCH (pg)	MCHC (gr/dl)	Monocytes (%)	Neutrophils (%)	Ascorbic Acid (mg/Kg)
0mg	101 ± 21.0	67.6 ± 5.4	16.1 ± 2.6	6.3 ± 0.49	299.31 ± 21.1	52.6 ± 6.3	17.5 ± 1.6	99 ± 1.08	1.06 ± 0.14	≤ 0.01
10mg	97 ± 16.2	69.6 ± 8.9	33.3 ± 2.8	6.5 ± 0.42	289.21± 38.2	56.4 ± 7.1	19.5 ± 0.2	99.5 ± 0.58	0.67 ± 0.57	10.5 ± 0.13
20mg	102 ± 21.5	69.3 ± 8.5	31.6 ± 1.1	5.7 ± 1.2	303.81± 18.7	59.1 ± 6.2	17.9 ± 2.2	98 ± 1.03	1.22 ± 0.16	18.23±0.63
30mg	103 ± 10.3	64.3 ± 6.8	28.3 ± 1.5	5.9 ± 0.13	316.12 ± 49.5	59.5 ± 8.3	18.8 ± 0.3	98.6 ± 1.5	1.37 ± 0.53	28.29±1.63
40mg	103 ± 12.7	64.5 ± 16.2	32.3 ± 1.5	5.9 ± 0.81	244.45± 11.5	51.1 ± 6.7	20.9 ± 2.4	99.6 ± 0.58	0.33 ± 0.05	37.52±1.08
80mg	105 ± 21.7	63.6 ± 15.5	34.6 ± 1.1	6.1 ± 0.11	300.46 ± 25.7	56.1 ± 8.7	18.6 ± 1.8	98.3 ± 2.08	1.26± 0.73	56.85±0.5
120mg	108 ± 18.5	61.8±12.8	36.21± 1.2	6.9 ± 1.23	315.61± 22.6	55.3±5.4	21.6±1.24	99.4 ±1.28	0.57±0.24	116.18± 0.8

Values are Mean ± SD of six individual observations. Mean values are significant at $p < 0.05$.

IV. DISCUSSION

Ascorbic acid is an important intracellular antioxidant and is involved in the self-defence mechanisms of fish. In the present study fishes were fed with vitamin C enriched diet for 8weeks. Vitamin C is an important intracellular antioxidant and is involved in the self-defence mechanisms of fish. It works as an antitoxic agent against heavy metals[38], pesticides[39] and microbial assaults in fish[40]. It is involved in intermediary metabolism.

The results of the present study strongly suggesting that vitamin C significantly influence on the growth, survival and haematology of juvenile *Catla catla*. Growth is a function of both the nutritional quality and the rate of consumption, among other things[41].

In the present investigation Vitamin C influenced marked variations in all parameters with increase of concentration. Similarly, studies with common carp[42], hybrid tilapia, *Oreochromis niloticus*[43], Japanese seabass, *Lateolabrax japonicus*[44], juvenile grouper, *Epinephelus malabaricus*[45] and juvenile cobia, *Rachycentron canadum*[46] indicate positive effect of vitamin C on the growth.

In the present study, a diet containing 80mg of ascorbic acid kg^{-1} diet was found to be the optimal dietary requirement for juvenile *Catla catla* while 120mg ascorbic acid kg^{-1} diet was found to be the requirement level for maximum growth and performance of *Catla catla*. The requirement value of ascorbic acid of *Catla catla* accomplished in the present study was higher than reported for *Oreochromis aureus* between 10 and 25 mg ascorbic acid and who also examined juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*[46]. The difference might be varied species to species, size, the form of vitamin C and experimental conditions of different studies[47]**.** Weight gain increased with varied dietary level is considered by majority of nutritionists to be the most important and consequential response in nutritional studies[48]. The diet without ascorbic acid supplementation decreased the specific growth rate (0.32 % day-1, control - 0mg AA) of juvenile *Catla catla*. Ai *et al.*[49] also observed declining specific growth rate with ascorbic acid deficient diet for sea bass (*Scophthalmus maximus*). Stickney[50] reported that feed conversion factor values can actually be less than 1 in water systems where they feed on natural food. Goddard[51] distinguished that alternating feed conversion ratios may reflect problems with diets or feeding methods. In the present study, fish fed with non supplemented diet had poor food conversion ratio (12.5) while fish fed with the supplemented diet recorded 21.25 at 120mg as feed conversion ratios.

Feed utilization in this study was also affected by the varying dietary level of ascorbic acid. Total amount of feed consumed increased with increased ascorbic acid level. Both protein conversion efficiency and protein efficiency ratio were much lower in fish fed with diet without ascorbic acid. This indicate lower protein utilization by the fish[52, 53]**.** Fracalossi *et al.*[54] observed a similar trend in juvenile Oscars, *Astronotus ocellatus*. The diet used in the present experiment had high protein content (400 g kg-1), which could have resulted in the fish consuming high levels of oxygen as consumption increases with protein in fish[55].

In this study, a number of other healthy related conditions were uncovered due to ascorbic acid deficiency. For instance, haematology of the fish was significantly affected by ascorbic acid. Fish without ascorbic acid supplementation showed lower values for hematocrit (16%), total white blood cell (67.6 $x10^3/mm^3$), red blood cells ($101x10^6$ $/mm^3$) and plasma protein (1.56 g/dl). These negative trends compromised the healthy status of the fish that showed clinical signs such as fin erosion and broken back disease. Shiau and Jan [53] also reported that anaemia is common in fish of ascorbic acid deficiency because there is reduction in the absorption and redistribution of iron and consequently a reduction in the synthesis of hemoglobin. Fracalossi *et al.*[54] also reported reduced growth, impaired collagen formation and lordosis (deformities) in *Astronotus ocellatus*, due to deficiency of ascorbic acid. In this study, juvenile *Catla catla,* do not supplemented ascorbic acid caused elevated levels of neutrophils. Roberts[57] reported the excess release of neutrophils into the blood of fish is in response to stress. Deficiency of ascorbic acid may have induced a physiological stress on juvenile *Catla catla*. It is evident that there is a relationship between tissue ascorbate and the fish health. In the present study fish fed with Vitamin C non supplemented diet showed declining ascorbate levels in both the liver and the muscle. On the other hand, fish with Vitamin C supplemented diet caused to increase the level of ascorbate. Foroushany *et al*.[58] reported that requirement for tissue saturation is much higher than that for normal fish growth that could prevent deficiency signs. Therefore increased tissue ascorbate in *Catla catla* fed with supplemented diet of ascorbic acid had a positive effect on the health of the fish[56]. A relatively low rate of survival (60%) was observed in juvenile *Catla catla* fed with a non-supplemented diet of ascorbic acid. The significantly lower survival in fish fed with deficiency diet of ascorbic acid can only be attributed to the physiological stress caused by dietary ascorbic acid deficiency and not due to water quality since all low water quality parameters were within the ranges known to be required.

Total erythrocyte count and haemoglobin concentration are considered as health indicators for fishes. In the present study the increase of erythrocyte count in Vitamin C treated fish indicating positive health effect on fishes. Previous studies have also shown that immunostimulants could increase immune functions by affecting the blood cells[56]**.** The RBC count of the group fed 80 mg and 420mg Vitamin C kg^{-1} feed was higher than other supplemented diet groups after 8weeks of feeding (Table 4). Haemoglobin in blood plays a vital role to carryout oxygen to deeper tissues. Haemoglobin concentration of Vitamin C treated group was significantly higher than the control group (Table 4). The primary study has indicating that Vitamin C caused to increase the oxygen supply in the blood of the fishes reflecting beneficial effect on the health of the fishes including *Catla catla.*

V. CONCLUSION

In conclusion, supplementation of 60mg ascorbic acid kg^{-1} in the diet significantly yielded the maximum fish weight gain and overall health condition is dependent on nutrient dose-response experiment. In the present study also we conclude that significant variations are observed in fish, juvenile *Catla catla*, such as growth, haematological parameters, protein levels and ascorbate levels fed with various levels of ascorbic acid.

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