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Research Article

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EFFECTS OF DIETARY VITAMIN-C ON BIOCHEMICAL AND MORPHOMETRIC PARAMETERS OF *LABEO ROHITA*

Ashok Kumar[1*], A. K. Bajpeyee[2] and Chandra Bhan Yadav[2]

[1]Department of Zoology, M. L. K. P. G. College Balrampur (U.P.), India
[2]Centre of Biotechnology, University of Allahabad, Prayagraj (U.P.), India
**Corresponding author:* abmlk1515@gmail.com

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Abstract: The major carp, *Labeo rohita* fed with six different diets having vitamin C fortification. The Halver's synthetic diet (H440) was supplemented with graded levels of vitamin C at 0, 250, 500, 600, 750 and 1000 mg/kg. In present investigation, *Labeo rohita* fed with 600 mg/kg vitamin C, gain maximum ash (4.16%), carbohydrate (2.94%) protein (16.87%), fat (5.09 %), SGR (2.92 %), survival rate (100 %) with least moisture content (77.10 %) as compared to control and other graded level of vitamin C. Supplementation of vitamin C beyond 600 mg resulted in decrease in protein, fat, carbohydrate and ash values with an increase in moisture content. The results also indicated that Halver's synthetic diet of fish without dietary vitamin C had lower weight gain (WG) and specific growth rate (SGR) than those fed the diets supplemented vitamin C. WG and SGR did increase with dietary vitamin C levels increasing from 250 mg/ kg to 600 mg / kg. However, no significant increase was observed with further increase in dietary vitamin C levels beyond 600 mg / kg.

Keywords: *Labeo rohita*, Synthetic diet, Vitamin C supplement.

INTRODUCTION

Vitamin C is an essential nutrient for the growth, development and repair of all body tissues. It is involved in collagen formation, iron absorption, maintenance of cartilage and bones. Ascorbic acid stimulates and activates the immune system by reimbursing the immune-depression and wound healing. The vitamin C protects fish by stimulating the activity of immune cells *i.e.* neutrophils and phagocytes. It is a strong reducing agent hence readily oxidized and acts as a co-factor for the hydroxylation of certain amino acids like proline, lysine and so on (Vélez-Alavez *et al.*, 2014). The hydroxylation of proline and lysine to hydroxyl proline and hydroxyl lysine in presence of propyl hydroxylase and lysyl hydroxylase stabilizes the collagen by cross linking of peptide in collagen. Subsequently collagen inhibits bacteria and viruses. Vitamin C also prevents bacteria from adhering to epithelial cells due to lack of L-gulonolactone oxidase enzyme.

The biosynthesis of vitamin C does not occur in fishes except two to three species. Vitamin C is

one of the strongest antioxidant (Rani *et al.,* 2019) defensive systems in fishes associated with vitamin E and antioxidant enzymes to strengthen the body's defence mechanism. Fishes are exclusively aquatic and cold blooded animals with streamlined body and lateral line sense organs (Verma and Prakash, 2020). They have either cartilaginous or bony endoskeleton. The fish studied is a bony fish.

Although extensive works on toxic impact of metals on the haematological, biochemical and histological parametres of fish have already been carried out (Srivastava and Prakash, 2019; Kumar *et al.,* 2019; Prakash and Verma, 2020) but till now there is no sufficient baseline data about impact of vitamins on growth and development of fishes, hence authors have undertaken to study the impact of vitamin C on biochemical and morphometric parameters of Indian major carp, *Labeo rohita*.

MATERIALS AND METHODS

The *Labeo rohita* of 3.6 cm (approximately) in *length* and weight 7±0.23 mg (approx.) were procured from local fish nursery of Balrampur, Uttar Pradesh. After one week of acclimatization, fishes were randomly distributed into 18 plastic tanks (capacity of 50 L) at the density of 10 tank^{-1} at three replicates for each experimental diet with continuous aeration and exchange of water on alternate day. The Halver's synthetic diet (H440; Halver, 1980) was supplemented with graded levels of vitamin C 0, 250, 500, 600, 750 and 1000 mg/kg. Experimental diet was fed three times daily for 60 days. During experimental period, fishes were maintained under photoperiod of 12:12 (light: dark). Water temperature was kept at 25.5 ± 1.5°C throughout feeding trail. Dissolved oxygen (6.53 ±0.21 mg L^{-1}) and pH (7.64 ± 0.14) were maintained and measured every week. This physico-chemical parameter of experimental water was analyzed by APHA (2005). Moisture content was determined using the hot air oven, by drying the sample at 105 °C for 12h.

Biochemical composition of *Labeo rohita* such as crude protein content was determined by converting the nitrogen content with the help of Kjeldahl's method (Nx6.25), lipid was calculated gravimetrically after extraction with petroleum ether in a soxhlet system (SOCS, Pelican, India), ash content was determined after combustion for 6 h at 600 °C in muffle furnace and carbohydrate was determined by subtracting the sum of fat content, protein content, ash content and moisture from 1000 (AOAC, 1990).

RESULTS AND DISCUSSION

In the present exploration, authors recorded the maximum ash (4.16%), carbohydrate (2.94%) protein (16.87%), fat (5.09 %), SGR (2.92 %) with 100 % survival rate and least (77.10 %) moisture content in major carp fed with 600 mg/kg vitamin C, as compared to control and other graded level of vitamin C. Authors also noticed that the supplementation of vitamin C beyond 600 mg led in decrease of protein, fat, carbohydrate and ash values with an increase in moisture content. The diet devoid of vitamin C resulted in least %age of protein, fat, carbohydrate and ash with highest level of moisture, as shown in table 1-2.

Table 1: Effect of graded levels of vitamin C on biochemical composition of *Labeo rohita*.

Diet. No.	Vit. C mg/kg	Biochemical parameters (in percentage)				
		Ash	Carbohydrate	Protein	Fat	Moisture
Initial	----	3.54±0.30	1.77±0.60	12.95±1.2	1.86±0.20	86.01±2.50
Diet -1	00	3.77±0.50	2.35±0.51	14.19±1.36	2.22±0.14	84.30±2.10
Diet-2	250	3.84±0.10	2.43±0.21	14.82±1.27	3.78±0.33	82.50±2.19
Diet-3	500	4.02±0.20	2.73±0.71	15.98±1.09	4.99±0.28	79.44±2.07
Diet-4	600	4.16±0.15	2.94±0.12	16.87±1.08	5.09±0.18	77.10±2.80
Diet-5	750	3.93±0.11	2.89±0.31	15.40±1.18	4.92±0.51	78.08±1.90
Diet-6	1000	3.46±0.19	2.66±0.25	15.00±1.23	4.36±0.14	76.20±1.50

The effect of vitamin-C on growth, survival time of spawning, development and immune response was studied by some workers including Ai *et al.* (2004, 2006). The moisture content of the muscles of fish plays a major role in its metabolism because for all biochemical reaction water is required. The water content of fish bodies varies within a limited range in different species. In the present study, the percentage of moisture was decreased with the increase in supplementation quantity of vitamin C up to 600 mg/kg level, beyond that an increasing trend was observed.

Table 2: Effect of graded levels of vitamin C on morphometric parameter of *Labeo rohita*.

Morphometric parameters of fish	Doses of vitamin C with Halver's synthetic diet All values are means of three replicates ± SE					
	Control	250 mg/kg	500 mg/kg	600mg /kg	750 mg/kg	1000 mg/kg
Initial weight (g)	7.12±0.10	7.16± 0.04	7.23± 0.17	7.15± 0.06	7.18±0.17	7.22±1.9
Final weight (g)	15.05±0.20	15.69±0.32	16.47±0.12	20.32±0.45	17.23±0.33	16.10±0.2
Weight gain (g)	9.93± 0.04	10.53±0.23	11.24±0.76	15.17±0.52	12.05±0.50	11.5±0.10
SGR (%)	2.15± 0.20	2.24±0.10	2.33± 0.18	2.92±0.085	2.48±0.15	2.07±0.10
Survival rate (%)	86.33± 2.5	90± 1.20	93.33± 1.5	100± 0.50	96.66± 1.0	95.42±0.12

Authors found an inverse relationship between moisture and fat in *Labeo rohita* fed on diet 4 (600 mg/kg). It showed maximum protein accumulation from 12.95% at the beginning to 16.87% in diet pattern 4 that correlates with the findings of Halver *et al.* (1972).

Vitamin C is one of the strongest antioxidant defense systems in fish. It acts against intracellular and extracellular reactive oxygen species. Phagocytes, major actors of the innate immune response of fish, contain a high concentration of vitamin C in their cytoplasm; this represents strong protection against the huge production of reactive oxygen species in fighting against pathogens. The combination of vitamins C and E in fish contributes to improve growth, immune response, metabolism of nutrients and resistance to stress; vitamin C aids in the process of absorption of vitamin E and metabolism of lipids (Corredor and Landines, 2009). Vitamin E concentration increased in the muscle, liver, heart and kidney of *S. maximus* (turbot) receiving diets with high in α-tocopherol, while the effect on the concentration of ascorbate in muscle was the opposite (Ruff *et al.*,2003). Fish fed the lowest vitamin C diet had lower weight gain (WG) and specific growth rate (SGR) than those fed the diets supplemented with vitamin C. WG and SGR did significantly increase with dietary vitamin C levels increasing from 1.9 to 156.5 mg kg^{-1}. However, no significant increase was observed with further dietary vitamin C levels increasing from 156.5 to 316 mg kg^{-1} (Liang *et al*,, 2015).

Based on the different combination of experimental results obtained in the present study it can be concluded that mixing of vitamin C (maximum 600 mg/kg of fish diet) will be helpful for the growth and development of fry, fingerlings and adults.

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