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Use of vitamin C as an immunostimulant. Effect on growth, nutritional quality, and immune response of *Labeo rohita* (Ham.)

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Abstract Teleost fish lack the enzyme for endogenous synthesis of ascorbic acid (AA), an essential micronutrient for fish. The aim of this study was to examine the effect of higher levels of dietary vitamin C on growth, nutritional quality, and immunomodulation in the Indian major carp, rohu (*Labeo rohita*). Four groups of *L. rohita* were fed experimental diets containing either no vitamin C (control) or supplemented with vitamin C at 500 mg kg^{-1} (Exp-1), 1000 mg kg^{-1} (Exp-2), or 1500 mg kg^{-1} (Exp-3) for 60 days. Growth parameters (NWG, ADG, and SGR), serological parameters (TSP, TSA, TSG, and A:G), haematological parameters (TLC, TEC, Hct, MCV, and MCH), and different non-specific immunological parameters (PR, PI, respiratory burst activity, and bactericidal activity) were evaluated during the experimental trial. Fish fed a vitamin C-supplemented diet showed higher specific growth rate (SGR) up to 1000 mg kg^{-1} compared with control fish. Different haematological and serological parameters along with non-specific immune parameters were influenced by vitamin C supplementation. Among the non-specific immune parameters phagocytic activity (PR and PI) and respiratory burst activity (NBT cells) were significantly ($P \leq 0.05$) enhanced by increasing doses of vitamin C supplementation. Higher levels of dietary vitamin C significantly ($P \leq 0.05$) enhanced protection against *Aeromonas hydrophila* (AH1) infection compared with controls. Results from this study help to establish the beneficial effect of vitamin C on growth and immunmodulation in rohu (*L. rohita*).

Keywords Growth · Immunomodulator · *Labeo rohita* · Vitamin C

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Introduction

Vitamin C (ascorbic acid, AA) is an important antioxidant vitamin and intake of vitamin C has been correlated with health in humans, animals, and cultured cells. In fish also it is assumed that vitamin C is an essential nutrient for optimum growth and maintenance (Dupee 1966; Halver et al. 1969; Lovell 1973; Mazik et al. 1987). With the exception of, perhaps, two or three species, vitamin C biosynthesis does not occur in fish due to the lack of the last enzyme of the biosynthetic pathway—L-gulonolactone oxidase. The major signs of ascorbate deficiency include reduced growth, scoliosis, lordosis, internal and fin haemorrhage, fin erosion, and increased mortality. Another beneficial effect of vitamin C, which has been established in a number of animal species, including fish, is to stimulate the non-specific immune response. The exact magnitude of vitamin

C-related disorders in aquaculture is not known, but vitamin C deficiency has caused significant losses in practical fish farming, especially during the sensitive start period.

Although a few data suggest that the use of dietary vitamin C in fish improves their natural resistance to infections, the possible mechanism and the doses are not well established. In the Indian major carp, in particular, the results are too scanty. So, the aim of this study was to establish the effects of dietary vitamin C in common fish diets on the Indian major carp (*Labeo rohita* Ham) non-specific immune system, paying special attention to the dose of the immunostimulant and the timing necessary to elicit an enhanced immune response.

Material and methods

Fingerlings of the species were obtained from a carp culture farm in the vicinity of the university campus. Fish (5.8 ± 0.2 g) were released into continuous flow glass aquaria ($76 \times 41 \times 41$ cm^3 volume; 200.0 L water-holding capacity) and acclimatized to prevailing laboratory conditions for 15 days. Studies were conducted at room temperature for 60 days. The water quality (temperature pH, dissolve oxygen, alkalinity, ammonia) of the experimental aquaria was monitored periodically as stated by the APHA (1998) and maintained at normal level.

Preparation of experimental feed

The four prepared types of feed (Control, Exp-1, Exp-2, and Exp-3) were formulated using locally available ingredients (mustard oil cake, rice polish, fish meal, and tapioca powder). Feed was formulated basically by the "square method" using determined values of the protein content of the different ingredients. Vitamin C (AA), available from Ranbaxy Fine Chemicals, India (Product no. A2840) was used as an immunomodulator at levels of 500, 1000, and 1500 mg kg^{-1} in experimental feed Exp-1, Exp-2, and Exp-3, respectively, whereas control feed was not supplemented with vitamin C. Pellets were prepared by use of a pelletizer, dried in a thermostatically controlled hot air oven at 37°C, and kept at less than 10% moisture (Bazaz and Keshavanath 1993; Keshavanath and Renuka 1998) stored in an air-tight jars at room temperature.

Growth performance and conversion ratio

Fish were fed twice daily at 8.00 and 16.00 h with ration size maintained with 6% of their body weight in two equal portions. The net weight increment was recorded every 15th day with an electronic balance and the quantity of feed was readjusted after this 15-day interval. For evaluating dietary performance, the nutritional indices live weight gain (LWG), net weight gain (NWG), average daily growth (ADG), feed conversion ratio (FCR), specific growth rate per unit (SGR), and protein efficiency ratio (PER) were calculated. Two fish from each treatment were sacrificed by overdose anaesthetisation with MS222 (Sigma Chemicals, India) (Bandyopadhyay et al. 2005) at the end of the experiment and stored at –20°C until analysis of these parameters.

Proximate analysis

Proximate analyses of feed ingredients, feed, and body carcass were determined by the AOAC (1990) method. Moisture content was determined gravimetrically in a hot air oven at 100 ± 10°C for 24 h. Crude protein content was determined by the micro-Kjeldahl method. Crude lipid was estimated by extraction with petroleum ether (boiling point: 40–60°C) in an electro-thermal Soxhlet apparatus. After extraction of the lipid the defatted samples were used for estimation of crude fibre following Patra (2002). Ash content was estimated by igniting samples in a muffle furnace (Instrumentation India, Kolkata, India) at 500 ± 50°C for 10 h.

Biochemical analysis

The deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content of the liver (hepatopancreas) tissues were estimated by the method of Munro and Fleck (1969). Tissue was taken from the fish and homogenized with 0.25 mol L^{-1} sucrose solutions. Homogenate (250 μL) and 5% TCA (500 μL) were mixed thoroughly, centrifuged, and, after waiting for

15 min, the precipitate was dissolved in PCA (several times at different concentrations) and centrifuged at 5000 rpm (twice). The ultimate supernatant was used for RNA and DNA estimation by UV spectrophotometry. GOT and GPT activity in the liver were determined by following the method of Bernfeld (1955).

Study of blood parameters

Blood samples for haematological study were collected from the caudal vein by means of a heparinized syringe. Ethylene diamine tetraacetic acid (EDTA) was used as anticoagulant. EDTA (1.0 mg mL^{-1} blood or one drop of 1.0% solution per 5 mL^{-1} blood) was used for haematology and the different parameters were estimated according to the method of Wintrobe (1978). MCV and MCH were calculated by using the standard formulae of Decie and Lewis (1991). Blood samples from the caudal vein and heart were collected in the laboratory for serological diagnosis. Total serum protein (TSP) and albumin (TSA) were determined by Gornall's biuret method (Kulow 1967). The globulin content is the difference between total proteins and albumins.

Determination of immunity level

On day 60, blood was collected from the fish of each experimental group by use of a 0.2-mL glass syringe rinsed with an anticoagulant. Part of the blood was heparinised and the rest was allowed to clot for serum samples, which were preserved at –20°C for further analysis. Immediately after collection the heparinised blood samples from each group were pooled into three aliquots. The rest of the heparinised blood was immediately used for the phagocytic assay (Siwicki et al. 1994; Park and Jeong 1996). Freshly prepared 0.1 mL NBT solution was added to 0.1 mL heparin mixed blood and 15 μL stimulant solution in an incubating bottle. The bottles were incubated at 37°C for 10 min and at 26°C for another 10 min. This blood (50–70 μL) was transferred to a clean slide and a thick smear was made with a spreader slide. The slides were air dried, stained with Wright's stain, and studied under an oil-immersion lens at 100×. Positive cells had violet formazan granules in the cytoplasm. The percentage of positive cells gave an idea about the non-specific immune status of the organism. Plasma (100 μL) and bacterial suspension (*Aeromonus hydrophila* (AH1) sub sp.; 10^4 CFU mL^{-1}, 100 μL) were mixed and incubated for 1 h at 25°C for determination of bactericidal activity by the method of Leano et al. (2003).

Challenge trial

Rohu fish (*L. rohita*) of medium size (435 ± 28 g) were collected from a semi-intensive culture pond at the university campus. The intestines were gently excised and cut open with a pair of sterile scissors. The non-adherent micro flora of the intestine were isolated by washing three times with sterile solution and homogenized with 10 mL distilled water in stomacher bags. The presumptive numbers of micro flora were determined by the spread plate technique using nutrient agar. The pathogenic strain *A. hydrophila* (AH1) was isolated by the method of Kaneko (1971) and cultured and maintained in *Aeromonas* selective medium (M884, Hi-Media).

After 60 days feeding trial, fish of each experimental group were released in four aseptic tanks. Water quality-parameters (temperature pH, dissolve oxygen, alkalinity, ammonia) were maintained at normal levels. Different experimental feed and control feed were provided twice daily in accordance with 6% of their body weight. The fish in each treatment were challenged with *A. hydrophila* (AH1). Fish in all replicates were immersed in a suspension of *A. hydrophila* (AH1), $\sim 10^5$ CFU mL^{-1} according to Austin et al. (1995). This was followed by a second immersion $\sim 10^7$ CFU mL^{-1} after 7 days (Austin et al. 1995). Survival of the fish against the pathogenic strain was recorded for 10 days.

Statistical analysis

As all the above analyses were carried out on pooled samples of a given lot, standard deviations of means were calculated. However, for evaluating dietary performance, nutritional indices, enzymatic activity and RNA:DNA ratio, different haematological, serological, and immunological parameters, and challenge trials, correlation and regression tests were

performed by use of the SPSS software package. Significant differences between the means of the treatments were tested by use of the Duncan multiple range test (Duncan 1955) by use of the SAS software package (1991).

Results

The approximate amounts of the different ingredients used to prepare experimental feed for *L. rohita* are presented in Table 1. The crude protein percentages of mustard oil cake, rice polish, and fish meal were 39.23, 13.03, and 48.65 respectively, whereas, the crude lipid percentages were 12.24, 5.14, and 6.72 respectively. The three experimental feeds (Exp-1, Exp-2 and Exp-3), but not the control feed (Control), were supplemented with vitamin C. All four experimental feeds were isocaloric and isonitrogenous.

Initial and final carcass composition of *L. rohita* in relation to the various diets are presented in Table 2. Carcass analysis of the experimental fish showed significantly ($P \leq 0.05$) highest crude protein in feed Exp-2-fed fish (64.75 ± 0. 012) and lowest in control feed-fed fish (57.92 ± 0.024) and lipid percentage ranged between 15.72 ± 0.051 and 16.96 ± 0.074 (Table 2). This clearly indicates enhancement of carcass composition quality with increasing supplementation of vitamin C up to a specific level (1000 mg), perhaps due to the properties of the antioxidant and the ability to prevent peroxidation of unsaturated fatty acids. Strong correlation of SGR and ADG with protein and lipid in rohu larvae in different groups demonstrate the different growth rate and different anabolic activity due to feeding different concentrations of vitamin C in supplemented feeds. The growth-maintaining activity of AA is a specific effect related to the process of tissue formation. AA is required during the formation of collagen (the principle component of connective tissue), the organic substances of the exoskeleton, and the ground substances between cells (Jaffre 1984; Chen and Chang 1994). AA plays an important role in certain aspects of protein metabolism (Chatterjee 1967; Shiau and Jans 1992) and it is an essential molecule in the overall health of animals.

Growth of *L. rohita* in relation to the various feeds is presented in Table 3 and Fig. 1. The tables and figures indicate that maximum growth was observed for feed Exp-2-fed fish (50.88 ± 0.18) while the lowest growth was observed for control feed-fed fish (30.83 ± 0.12). Growth in terms of ADG was significantly ($P \leq 0.05$) higher (0.84 ± 0.018) in Exp-2 feed-fed fish whereas it was least in control feed-fed

Table 1 Approximate amounts of the different ingredients used in the feed formulations

Ingredients	Proximate composition (%)					
	Moisture	Dry matter	Crude protein	Crude lipid	Ash	Energy (kJ g^{-1})
Mustard oilcake	6.05 ± 0.56	93.95 ± 1.62	39.23 ± 0.11	12.24 ± 0.06	9.55 ± 0.09	9.49 ± 0.08
Rice polish	4.38 ± 0.32	95.62 ± 2.83	13.03 ± 0.17	5.14 ± 0.21	21.41 ± 0.06	7.67 ± 0.03
Fish meal	2.30 ± 0.19	97.70 ± 0.87	48.65 ± 0.33	6.72 ± 0.14	11.98 ± 0.19	9.91 ± 0.10

Moisture and dry matter expressed as percentages of fresh weight; crude protein, crude lipid, and ash are expressed as percentages of dry matter

Each datum is a mean from five (5) separate determinations

Table 2 Initial and final carcass composition of fingerlings of *L. rohita* from the 60-day experimental trial of four diets

Proximate composition of feed	Experimental diets			
	Control	Exp-1	Exp-2	Exp-3
Crude protein (% dry wt)	57.92 ± 0.024[a]	62.18 ± 0.012[b]	64.75 ± 0.024[c]	63.32 ± 0.015[d]
Crude lipid (% dry wt)	15.72 ± 0.051[a]	16.47 ± 0.092[b]	16.96 ± 0.071[b]	16.59 ± 0.063[b]
Dry matter (% wet wt)	13.72 ± 0.03[a]	15.78 ± 0.03[b]	16.11 ± 0.02[b]	16.08 ± 0.01[b]

Figures having different letters (superscripted) in the same row are significantly different ($P \leq 0.05$)

Table 3 Different growth parameters, haematological parameters, and serological parameters

Diets	NWG (g)	ADG (g)	SGR (%)	FCR	PER	Hb (g %)	TLC ($\times 10^3$ mm $^{-3}$)	TEC ($\times 10^3$ mm $^{-3}$)	A:G ratio
Control	30.38 ± 0.12^a	0.50 ± 0.023^a	0.97 ± 0.032^a	6.10 ± 0.18^a	0.46 ± 0.01^a	7.1 ± 0.91^a	12.2 ± 0.76^a	1.04 ± 0.15^a	2.21 ± 0.021^a
Exp-1	33.82 ± 0.16^b	0.56 ± 0.024^a	1.22 ± 0.028^b	4.87 ± 0.16^b	0.62 ± 0.05^b	7.4 ± 0.17^a	12.8 ± 0.57^a	1.56 ± 0.11^b	2.11 ± 0.032^b
Exp-2	50.88 ± 0.18^c	0.84 ± 0.018^b	1.29 ± 0.019^c	3.43 ± 0.19^c	0.82 ± 0.04^c	7.9 ± 0.21^a	13.3 ± 0.76^a	1.72 ± 0.06^b	1.59 ± 0.025^c
Exp-3	39.86 ± 0.15^d	0.66 ± 0.019^c	1.19 ± 0. 017^b	5.90 ± 0.16^d	0.47 ± 0.05^a	7.5 ± 0.20^a	12.7 ± 0.87^a	1.43 ± 0.14^b	1.94 ± 0.014^d

Figures having different letters (superscripted) in the same column are significantly different ($P \leq 0.05$)

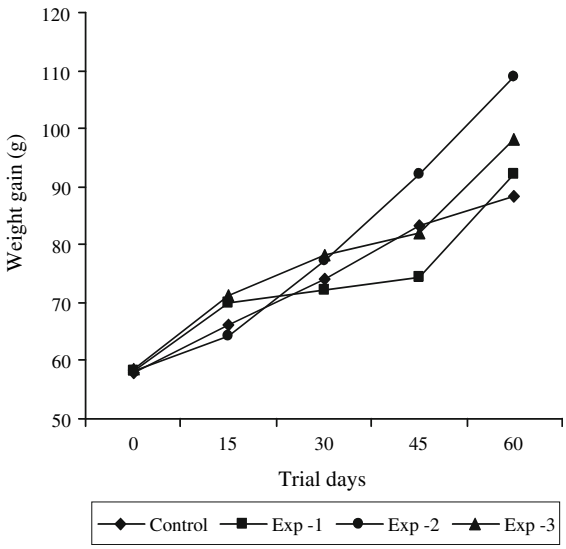

Fig. 1 Growth of *L. rohita* fingerlings in relation to different diets

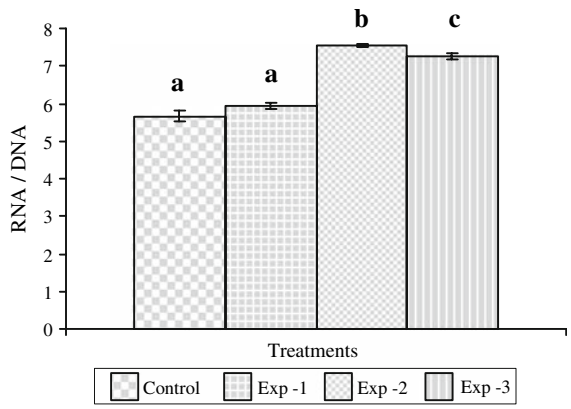

Fig. 2 Final RNA/DNA ratio in the muscle of *L. rohita* after the 60-day feeding trial

fish (0.50 ± 0.023). Significantly ($P \leq 0.05$) highest SGR and PER and lowest FCR were observed in feed Exp-2-fed *L. rohita*, whereas the lowest SGR and PER and highest FCR were observed in control feed-fed fish.

Significantly ($P \leq 0.05$) highest RNA:DNA ratio (7.55 ± 0.05) was observed for fish fed with Exp-2 feed and lowest was recorded (5.67 ± 0.15) for control feed-treated fishes (Fig. 2) after the 60-day feeding trial. It was also observed that the RNA:DNA ratio of fish increased in all the treatments over the initial RNA:DNA ratio (4.72 ± 0.03). The different levels of tissue protein and lipid deposition in different dietary treatments also support the above findings. Significantly ($P \leq 0.05$) highest GPT and GOT activity (ΔOD h^{-1} mg^{-1} protein) were observed in feed Exp-2-fed fish (0.048 ± 0.002 and 0.061 ± 0.003, respectively) whereas values were lowest for controls (Table 4).

Haematological values of *L. rohita* after the 60-day feeding trial are presented in Table 3. Significantly ($P \leq 0.05$) highest TLC, TEC, Hb, and Hct were observed in feed Exp-2-fed fishes where as significantly ($P \leq 0.05$) lowest TLC, TEC, Hb, and Hct was recorded in control feed-fed rohu fishes after the 60-day feeding trial. Blood is a patho-physiological reflector of the whole body and, therefore, blood parameters are important in diagnosing the status of fish (Pecie and Lewis 1991). In this study, all the blood parameters in all the treatments were similar to standard (Banerjee et al. 2002) and results were superior for feed Exp-2-fed fishes compared to other treated fishes and control fishes, which not only indicates the positive impact but also demonstrates the stable physiological reflection of the whole body (Pecie and Lewis 1991). In relation to different serological parameters TSP, TSA, and total serum globulin (TSG) were estimated to determine the effects of different feed on albumin:globulin ratio; the results are presented in Table 3. Highest albumin:globulin ratio was observed in control feed-fed

Table 4 Profile of GOT and GPT activity (ΔOD h^{-1} mg^{-1} protein) in the liver of *L. rohita* fed the experimental diets (Exp-1, Exp-2, and Exp-3) after the 60-day rearing period

Parameters	Diets			
	Initial value	Exp-1	Exp-2	Exp-3
GOT activity	0.034 ± 0.002[a]	0.043 ± 0.001[b]	0.048 ± 0.002[c]	0.046 ± 0.002[c]
GPT activity	0.048 ± 0.001[a]	0.055 ± 0.001[b]	0.061 ± 0.003[c]	0.065 ± 0.002[c]

Values with the same superscript in the same row are not significantly different ($P < 0.05$) from each other

GOT, glutamate-oxaloacetate transaminase (EC 2.6.1.1); GPT, glutamate-pyruvate transaminase (EC 2.6.1.2)

fish (2.21 ± 0.025) whereas it was lowest in Exp-1 feed-fed fish (1.59 ± 0.025) ($P \leq 0.05$).

The effects of vitamin C supplementation on non-specific immunity were observed in this experiment. Significantly ($P \leq 0.05$) highest phagocytic ratio (12.33 ± 1.65) and phagocytic index (2.34 ± 0.09) were recorded in feed Exp-2-fed fish whereas the lowest values (7.00 ± 1.42and 1.52 ± 0.12 respectively) were recorded for control feed-fed fish (Table 5). A similar trend was observed for number of NBT-positive cells. The significantly ($P \leq 0.05$) highest number of NBT-positive cells (42.81 ± 0.54) was observed in Exp-2 feed-treated fish and the lowest (12.05 ± 0.31) for control-feed-fed *L. rohita* (Table 4). However, the bactericidal activity of serum was lowest (6.00 ± 0.131 × 10^3 mL^{-1}) for fish fed Exp-2 feed whereas the highest bactericidal activity (7.11 ± 0.571 × 10^3 mL^{-1}) was recorded in control feed-fed fish.

After the 60-day feeding trial all treated and control fish faced a challenge trial with *A. hydrophila* (AH1) for 10 days. After the 10-day challenge trial survivability was significantly ($P \leq 0.05$) highest (93.33%) for Exp-2 feed-fed fish followed by Exp-3 (73.33%), Exp-1 (63.33%), and control (40%) feed-treated fish (Fig. 3).

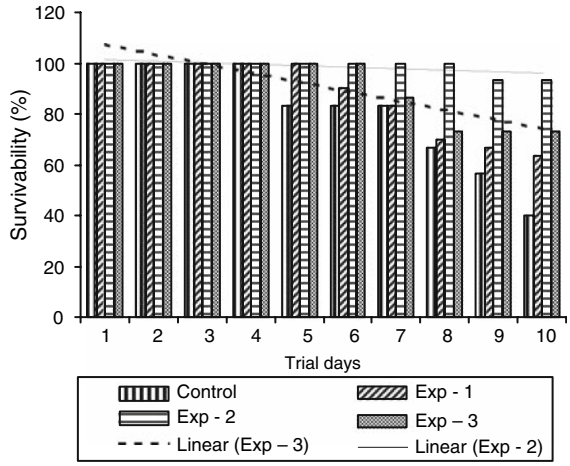

Fig. 3 Survivability of *L. rohita* in relation to various diets when challenged with *A. hydrophila* (AH1)

Discussion

In this study fish feed included vitamin C, which is thought to be useful for normal growth of fish. After the 60-day feeding trial a continuous growth trend was observed with no differences between the treated groups but with the treated groups significantly different from the control group. The growth-maintaining activity of AA is a specific effect related to

Table 5 Effect of vitamin C on phagocytic ratio, phagocytic index, and NBT positive cells (%) of *L. rohita* after the 60-day feeding trial

Treatment	Phagocytic ratio	Phagocytic index	NBT cells	Bactericidal activity (× 10^3 mL^{-1})
Control	7.00 ± 1.42[a]	1.52 ± 0.12[a]	12.05 ± 0.31[a]	7.11 ± 0.57[a]
Exp-1	9.12 ± 1.12[b]	2.15 ± 0.09[b]	27.33 ± 0.64[b]	6.34 ± 0.41[b]
Exp-2	12.33 ± 1.65[c]	2.34 ± 0.09[c]	42.81 ± 0.54[c]	6.00 ± 0.13[c]
Exp-3	11.15 ± 1.87[d]	1.49 ± 0.20[a]	39.43 ± 0.83[d]	6.28 ± 0.17[b]

Results are means ± SEM from five separate determinations

Figures having different letter superscripts in the same column are significantly different ($P \leq 0.05$)

the process of tissue formation; it is also required for collagen formation. Similar results were also observed by Mitra and Mukhopadhyay (2003) and Sahoo and Mukherjee (2003), who gave vitamin C to healthy and AFB_1-treated fishes. In the present study, although all the feeds were isonitrogenous, the concentration of vitamin C in Exp-2 feed might be helpful for proper nutrient utilization, because AA plays an important role in certain aspects of protein metabolism (Chatterjee 1967; Shiau and Jans 1992) and is an essential molecule in the overall health of animals. Whole-body carcass composition was higher for Exp-2 feed-fed fish than for control fish, which revealed the overall low feed-utilization level. Tissue RNA concentration and RNA:DNA ratios have been used as indicators of the recent growth or nutritional status of fish (Bulow et al. 1978; Miglavs and Jobling 1989; Mitra and Mukhopadhyay 2002). The RNA:DNA ratio indicates metabolic intensity which is deeply influenced by the nutritional status of the diet (Clemmensen 1987). The different RNA:DNA ratios between treatments in this experiment reflect the large extent of the effect of different AA levels on different anabolic activities. The different level of tissue protein and lipid deposition also supports the above result. The ratio was greatest in the fish fed Exp-2 feed, with higher dietary utilization and best growth. A similar result was also observed by Mitra and Mukhopadhyay (2003). The highest levels of GOT and GPT were found in Exp-2 feed-fed fish and the lowest in controls. But among the treated groups the level of GOT and GPT are not significantly different, which indicates that vitamin C does not affect GOT and GPT levels to a large extent.

Vitamin C not only influences growth and feed utilization but also has ability to be an immunomodulating agent. It affects the fish immune system, especially non-specific immunity, because fish seem to rely much more heavily on that immune system for protection (Landolt 1989; Roberts et al. 1995; Rougier et al. 1994). In this experiment vitamin C was supplied to fish, in feed, at a dose known to result in immunomodulation (Sahoo and Mukherjee 2003). Rohu, the species used in this study, is an important Indian major carp used in extensive, semi-intensive, and intensive fish farming throughout many tropical countries, including India. The dose of vitamin C used was based on earlier reports that high levels of vitamin C are able to stimulate the immune system of fish and also act as antioxidant vitamins; it has a high safety margin for dietary incorporation (Wagbo 1994; Sahoo et al. 1999). The use of dietary vitamin C to boost immunity in fish is controversial (Mulero et al. 1998). Despite conflicting results due to different experimental protocols and fish species, vitamin C has been demonstrated to modulate the immune response of fish when fed at a high level (Lal and Oliver 1993; Waagbo 1994). While some studies show a positive influence of vitamin C on macrophage activity (Blazer 1982; Verlhac et al. 1993; Verlhac and Gabuadan 1994; Mulero et al. 1998). Others have reported that phagocytosis, respiratory burst by macrophage and bactericidal activity are unaffected by dietary vitamin C (Hardie et al. 1991; Thompson et al. 1993). Under the current experimental conditions, the results suggested that vitamin C is able to enhance some non-specific immune parameters, such as bactericidal activity, phagocytic ratio, and respiratory burst activity, when compared to fish fed a basal diet, i.e. without vitamin C supplementation. Phagocytic activity in this study was strongly influenced by vitamin C, possibly due to their high demand for antioxidative substances necessary for preventing oxidative damage induced by free radicals produced to counteract pathogen aggression (Wahli et al. 1998).

Dietary vitamin C has been reported to have a positive effect on disease resistance (Durve et al. 1982; Navarre et al. 1989; Hardie et al. 1991; Waagbo et al. 1993). In the current study a high level of mortality due to *A. hydrophila* (AH1) infection was observed in fish fed the vitamin C-deficient diet (control feed) whereas the vitamin C-supplemented diets had a positive effect on survivability of the fish.

To conclude, these results suggest that a high vitamin C diet may increase growth and dietary utilization, and modulate the non-specific immune response (by enhancing phagocytosis-mediated leucocyte functions, bactericidal activity, etc.) and increase survivability against pathogenic agents. The results of this study indicate that high levels of vitamin C may be useful in field situations to counteract damage caused by pathogenic bacteria and other strains and thereby increase the general disease resistance of the animal.

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