

Evaluation of the Effects of Dietary Vitamin C, E and Zinc Supplementation on Growth Performances and Survival Rate of Rohu, *Labeo Rohita* (Hamilton, 1822)

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Abstract: Rohu, *Labeo rohita* is a popular variety of major carps for its highly nourishing quality and prolonged freshness. An experiment was undertaken to evaluate the effects of dietary vitamin C, E and zinc supplementation on growth and survivability of *Labeo rohita*. Four types of feed were used; Feed A as control feed, Feed B where basal feed supplemented with 100 mg kg⁻¹ vitamin C and E with 10 mg kg⁻¹ of zinc sulphate monohydrate, Feed C where basal feed supplemented with 500 mg kg⁻¹ vitamin C and E with 10 mg kg⁻¹ of zinc sulphate monohydrate, Feed D where basal feed supplemented with 1000 mg kg⁻¹ vitamin C and E with 10 mg kg⁻¹ of zinc sulphate monohydrate. One hundred twenty fish with an average weight of 225± 0.01 g were reared in twelve tanks to achieve four treatments with three replicates where each tank was provided with ten fishes. Significant increase in body weight increase (BWI), and specific growth rate (SGR) were observed in fishes supplemented with the feed C compared to the other groups of fishes fed with feed A, B and D. FCR and condition factor was also lowest when fed with feed C which was significantly different than the feed A, B and D thus indicating positive effects of feed C. Only survival rates were almost similar when *Labeo rohita* were fed with feed A (control feed) and feed C. Supplementation of vitamin C and vitamin E at 500 mg kg⁻¹ feed with 10 mg kg⁻¹ zinc might have effects on the growth performances of *Labeo rohita*.

Keywords: *Labeo rohita*, Growth performances, Survival, Vitamin C, Vitamin E, Zinc.

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I. Introduction

Fish has been playing a significant role in securing livelihoods and nutritional needs of the people in the developing countries. More than 50 percent of total animal protein intake in Small Island developing States, as well as in Bangladesh, Cambodia, Ghana, Indonesia, Sierra Leone and Sri Lanka was contributed by fish. Fish also contributed to about 17 percent of animal protein, and 6.7 percent of all protein consumed by global population. Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita intake of animal protein [1]. Species wise annual fish production in inland water in 2015-2016 was dominated by major carps by 23.7% and total major carp production was 750880 MT [2]. It is now essential to include vitamins and minerals in farmed fish feed for optimal growth and health. Vitamin C has been reported to function as general water soluble redox reagent, a cofactor in collagen formation, a regulator in steroid synthesis, a growth activator in wound healing, a modulator of the hexose monophosphate shunt and an activator of hepatic microsomal hydroxylases [3]. Vitamin C is an essential component of fish feed as fish cannot synthesize this vitamin due to the lack of enzyme L-gulonolactone oxidase [4]. Vitamin C might be present in natural food, but in intensive fish culture its supplementation becoming necessary. Vitamin E receives the most attention for its important role as an antioxidant. A number of studies have demonstrated that vitamin E is an essential supplement in fish feed for different fish species and noticeable effects on fish physiology [5]. Vitamin E has been reported to improve growth, stress and disease resistance and survivability of fish and shrimp [6]. Inclusion of Zinc (Zn) in fish feed is essential and is more absorbable than the waterborne Zn [7]. Zn acts as cofactor of several enzymes and incorporated with more or less 20 metalloenzymes such as alkaline phosphatase, alcohol dehydrogenase and carbonic anhydrase [8]. Zinc has several important roles including prostaglandin metabolism, nucleoproteins formation and controlling growth by zinc-gene interactions. Growth retardation is the result of zn deficiency. As essential component zinc is required for several biochemical processes in fish and the deficiency of zinc leads to growth impairment, mortality, dwarfism and low tissue zinc [9]. The aim of this study was to evaluate the effects of vitamin C, E and zinc supplementation on growth performances and survival rate of *Labeo rohita*.

II. Materials and Methods

2.1. Experimental procedure

2.1.1 Experimental design Four treatments with three replicates were used in this experiment. An indoor rearing system consisting of twelve plastic tanks was used for this purpose. Each tank supplied with almost 750L of water where *Labeo rohita* with mean weight range of 225 ± 0.01 g was stocked. Each tank provided with 10 fishes with 1:1 ratio of female and male. Before stocking the batch weight of fishes in each tank was also recorded to the nearest gram. Tap water was the source of water supply in the tanks and an adequate level of oxygen in each tank was maintained through artificial aeration by using air pump. About 30% of the water in each tank was exchanged daily.

2.1.2. Feeding trial

The experimental feeding trial was scheduled for a period of 4 months. The basal fish feed (Quality Feeds Limited) which consisted of fish meal, soybean meal, wheat flour, vegetable oil, soybean oil, salt, trash fish and vitamin mineral premix devoid of vitamin C, E and zinc was collected from local market near Central Veterinary hospital which were then formulated to achieve four supplemented feeds; Feed A as control feed, Feed B where basal feed supplemented with 100 mg kg^{-1} vitamin C and E with 10 mg kg^{-1} of zinc sulphate monohydrate, Feed C where basal feed supplemented with 500 mg kg^{-1} vitamin C and E with 10 mg kg^{-1} of zinc sulphate monohydrate, Feed D where basal feed supplemented with 1000 mg kg^{-1} vitamin C and E with 10 mg kg^{-1} of zinc sulphate monohydrate. Brand name of vitamin C was Pol C (Avon animal health), vitamin E was E vet (Acme Laboratories Ltd.) and zinc sulphate monohydrate was Zesup-vet (Square pharmaceuticals ltd.). Fish were fed two times a day with experimental feeds at a rate of 5% of the body weight per day during the whole experimental period. Batch weights of fish were taken every two weeks and feeding were adjusted accordingly.

2.1.3. Waste Water Removal

Feces in the tanks were cleaned every day morning by outlet before feeding commenced without creating any disturbances to fish.

2.1.4. Water quality management

Routine measurement for water quality (such as temperature, DO, pH etc.) was taken on a weekly basis.

2.2. Proximate composition analysis

The proximate compositions of each of the diets were done following the AOAC method [10]. Sampling was also carried out fortnightly.

2.3. Calculation

The following variables were calculated:

2.3.1 .Body Weight Increase

$$\text{Body weight increase (BWI)} = W_t - W_0 \quad [11]$$

2.3.2 Specific Growth Rate (SGR)

$$\text{Specific growth rate (SGR)} = (\ln W_t - \ln W_0) \times 100_{t-1} \quad [12]$$

2.3.3. Survival rate

$$\text{Survival rate} = N_t \times 100 N_{0-1} \quad [13]$$

2.3.4.Feed Conversion Ratio

$$\text{FCR} = \text{Feed given (dry weight)} / \text{Wet gain (wet weight)} \quad [14]$$

2.3.5.Condition Factor

Fulton's condition factor $K = W \times 100 / L^3$ [15] Where, W = weight of fish (g), L = Length of fish (cm), W_t and W_0 were final and initial fish weights (g) respectively; N_t and N_0 were final and initial numbers of fish in each replicate respectively; t is the experimental period in days.

2.4. Statistical Analysis:

Data was analyzed using one way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DMRT) to determine the differences between treatment means demonstrating a significant variation

of P value of <0.05 (at 95% confidence intervals) by using the statistical software package IBM SPSS Windows version 20.0 (IBM Corp.) Microsoft Office Excel 2010 was used to produce graphs.

III. Results and Discussions

Proximate compositions of the experimental feeds are presented in table 1. No significant differences were found among crude protein, lipid, ash, moisture and carbohydrate in different feeds.

Table-1: Proximate composition of the experimental diets (%)

Sl. No	Treatment Name	Crude protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Carbohydrate (%)
1	Feed A	36.01±0.11	10.18±0.03	11.6±0.01	11.70±0.05	30.51±0.14
2	Feed B	35.60±0.15	10.10±0.20	11.65±0.15	12.00±0.10	30.65±0.30
3	Feed C	36.45±0.08	9.80±0.15	11.35±0.07	11.20±0.10	31.20±0.33
4	Feed D	36.06±0.16	10.05±0.01	11.50±0.04	11.65±0.10	30.74±0.29

Significant increase in body weight increase (BWI), and specific growth rate (SGR) were observed in fishes supplemented with the feed C (figure 1 and figure 2 respectively) compared to the other groups of fishes fed with feed A, B and D. No significant differences were found on the survival rate of *Labeo rohita* when fed with feed B and feed C and moreover survival rate with feed A and feed D had no significant differences (figure 3).

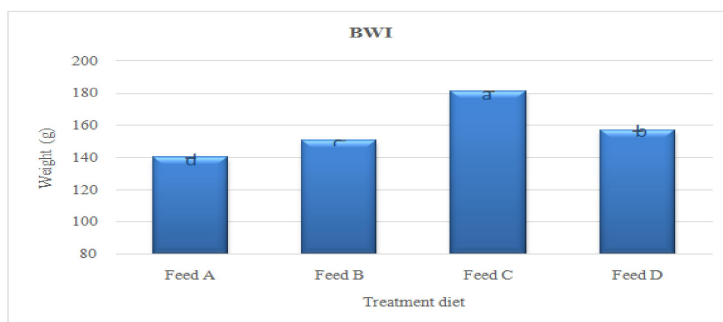


Fig. 1 Effects of vitamin C, E and zinc on body weight increase (BWI) of *Labeo rohita*.

However, interestingly significant differences were observed between feed A and D group with feed B and C groups. FCR of *Labeo rohita* was found to be lowest when fed with feed C which was significantly different than the feed A, B and D (figure 4). Condition factor of *Labeo rohita* fingerlings was lowest when fed with feed C and statistically significant differences were found between condition factor of *Labeo rohita* fingerlings fed with feed C and feed A, B and D (figure 5). During the study period BWI, SGR, FCR and condition factor of the rearing *Labeo rohita* showed results in favor of the use of formulated fish feed specifically diet with vitamin C and vitamin E at 500 mg kg⁻¹ feed with 10 mg kg⁻¹ zinc that was feed C. Only survival rates were almost similar when *Labeo rohita* were fed with feed A (control feed) and feed C.

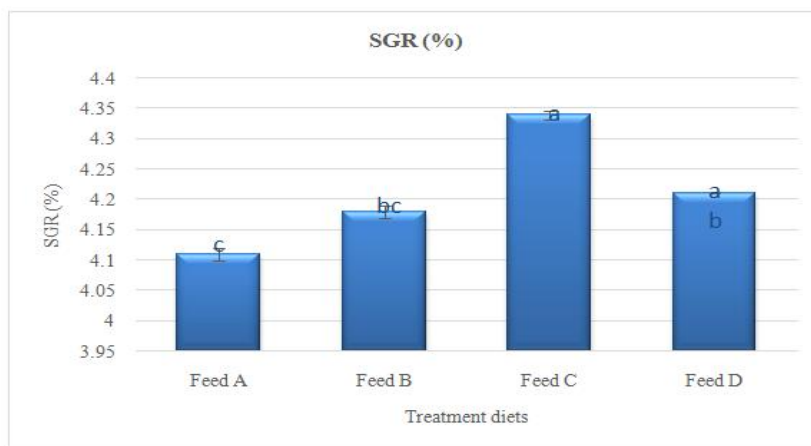


Fig. 2 Effects of vitamin C, E and zinc on specific growth rate (%) of *Labeo rohita*.

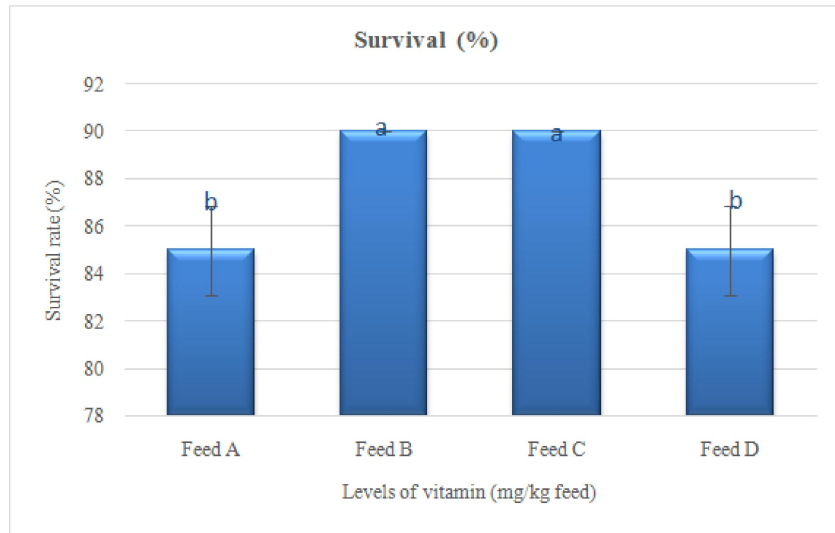


Fig. 3 Survival rate (%) of *Labeo rohita* fed on different formulated diets.

Studies on synergistic effects of vitamin C, E and zinc are very scarce. Synergistic effects of vitamin C at the rate of 1250 mg kg⁻¹, vitamin E at the rate of 600 mg kg⁻¹ and zinc at the rate of 120 mg kg⁻¹ resulted in increase of total number of spawns, higher mean clutch size, sperm viability, sperm motility, hatching rate and larval survival rate [15]. Combined effects of vitamin C and E in weight gain of juvenile hybrid tilapia [16] was reported. But such combined effects were not observed in case of fingerlings of *Labeo rohita* [15] and *O. niloticus* [17]. Other studies were done only on the sole effects of vitamin C or vitamin E or zinc which would be discussed here. SGR, FCR and survival rate of fingerlings of *Labeo rohita* were highest when fed with basal diet supplemented with 500 mg kg⁻¹ of vitamin C which agrees partially with the results of current study. However, optimum requirement of *Cirrhinus mrigala* was reported to be 700 mg kg⁻¹ [18]. Weight gain and SGR substantially increased with the increased level of vitamin C in common carp fries although PER was also significantly higher and FCR was lower when feed supplemented with 400 mg. No significant differences were observed for survival rate among the treatments [19]. Juvenile tilapia (*Oreochromis karongae*) when fed with vitamin C supplemented diet had significantly higher SGR, PER and than non supplemented fish [20]. Body weight gain, SGR and CF (condition factor) were significantly higher in benni fingerlings when fed with 1000 mg kg⁻¹ vitamin C supplemented feed than the lower vitamin C supplemented feed [21]. Similar results were observed including the significant increase in FCR and PER for *Labeo rohita* fingerlings [22] and for *Heteropneustes fossilis* [23].

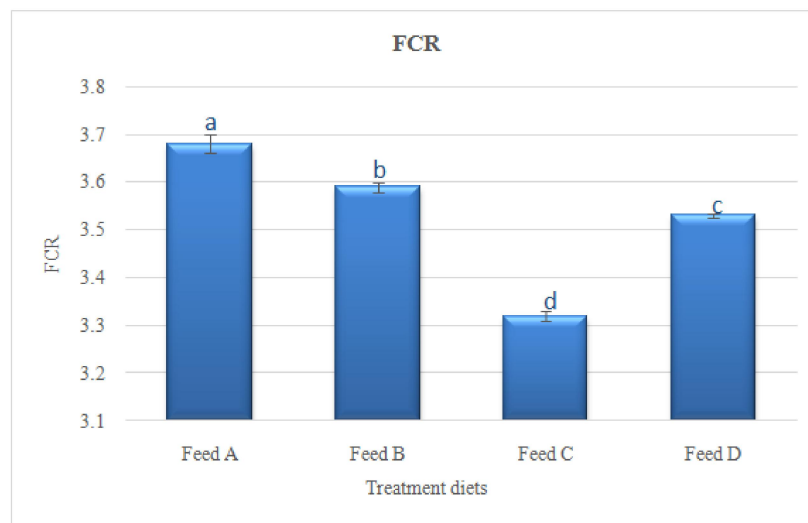


Fig. 4 Effects of vitamin C, E and zinc on feed conversion ratio (FCR) of *Labeo rohita*.

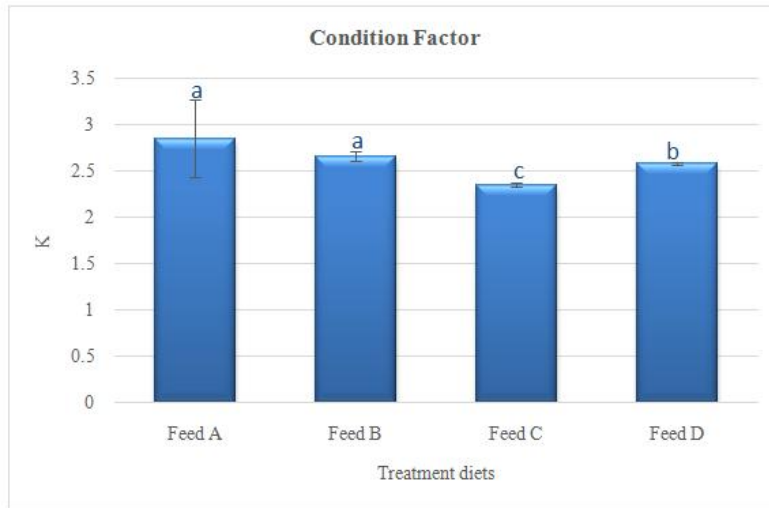


Fig. 5 Effects of vitamin C, E and zinc on Condition factor of *Labeo rohita*

However, no significant differences were observed in *Pseudosciaena crocea* [24] and *Clarius gariepinus* [25] when they were fed with diet supplemented with different levels of vitamin C. Survival rate of benni fingerlings were also reported to be increased [21]. These results might be due to the critical role of vitamin C in protein metabolism and normal physiological functions [21, 22, 26]. Highest level of growth and survival of common carp larvae were achieved when dietary ascorbic acid was given [27]. Despite the above mentioned facts, some authors found no significant differences on survival of *Heterobranchus longifillius* [28], *Clarius gariepinus* [25] and *Lateolabrax japonicas* [24] when fed with different levels of vitamin C. Requirements of vitamin C as supplement in fish feed may depend on various factors including stable forms of vitamin C [24, 29], metabolic rate [30], fish species and utilization of vitamin C [24, 31]. Vitamin C acts as essential coenzyme in different oxidative processes such as oxidation of tyrosine and phenylalanine and for this reason % body weight increased [19]. Growth might be influenced by vitamin C as it is responsible for the collagen formation which is essential for normal growth [32]. SGR, BWI and BWG were higher in Zebra fish supplemented with 500 mg kg⁻¹ vitamin E in feed than the control group [33]. Diets supplemented with 90 mg kg⁻¹ and 300 mg kg⁻¹ of vitamin E when fed to striped catfish (*Pangasius hypophthalmus*) had significant higher growth rate than the fishes that were fed only with farm processed diet [34] while significant weight gain and highest survival of catla (*Catla catla*) fry were accomplished when diet supplemented with 150 mg/kg of vitamin E [35]. It was found that measures of BWI was significantly improved when *Danio rerio* were fed a diet supplemented with vitamin E (1000 mg kg⁻¹) [33]. A similar result was also obtained by another author for Goldfish, *Carassius auratus* [36]. Vitamin E as supplement in feed has been suggested for rainbow trout 370 mg kg⁻¹ [37] and for channel catfish 100 mg kg⁻¹ [38]. Dietary vitamin E had no effects on growth of Indian major carp *Labeo rohita* [39], Atlantic salmon [40] and Channel catfish *Ictalurus punctatus* [41] which might be explained by the facts of adequate supplement of vitamin E in the diet components and regeneration capacity of alpha tocopherol by vitamin C [15]. Requirements for zinc in different fishes are different. Requirements for rainbow trout and salmon in fish feed are 15-30 mg kg⁻¹ diet and 80-118 mg kg⁻¹ diet respectively. White fish meal based diet supplemented with 40 mg zinc improved the growth of rainbow trout and carp [42]. Zinc requirements for channel catfish and rainbow trout in feed supplementation 200 mg kg⁻¹ and 150 mg kg⁻¹ respectively has been recommended [43] whereas 100 mg kg⁻¹ for Atlantic salmon [44]. Fish weighing 10 g were fed five diets containing organic zinc at 30, 70, 110 and 150 mg kg⁻¹ diet and one inorganic zinc source (zinc oxide) at 150 mg kg⁻¹ diet for a period of 12 weeks. No significant ($P > 0.05$) differences were found in the growth performance parameters such as final weight, specific growth rate [45].

IV. Conclusion

On the basis of the observations made during the rearing and feeding experiment of *Labeo rohita* fed on four different types of fish feed. It might be concluded that the formulated fish feed are found to be effective for the better growth of *Labeo rohita*. Fish feed containing adequate amount of vitamin E, vitamin C and Zinc supplementation might be the best feed for better growth and survival. Current study was completely laboratory based, so future field studies are necessary to determine the original effect of vitamin E, vitamin C and Zinc supplementation.

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