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Effect of Dietary Supplementation of Vitamin C on Growth Performance, Feed Utilization and Carcass Composition of *Barbus sharpeyi* Fingerlings

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Abstract

A study was conducted to determine the effects of dietary vitamin C on growth performance, feed utilization and carcass biochemical composition of *Barbus sharpeyi* fingerling. Five diets containing (0, 500, 1000, 1500 and 2000 mg kg^{-1}) of ascorbic acid were fed to benni fingerlings (6.96±0.3 g) in triplicate tanks and twice daily for a period of 56 days. The weight gain significantly increased (3.85 g) with enhanced feeding supplementation levels up to 1000 mg kg^{-1} diet, but no further increase was observed in the experimental treatments with feed supplementation levels, higher than 1000 mg kg^{-1}. Feed conversion ratio was significantly higher ($P<0.05$) in fish fed with control diet. A general increase in protein efficiency ratio was observed with increase in dietary vitamin C levels. Survival rate (%) did not display any significant difference among the experimental treatments. The body protein content increased significantly with the increase in dietary ascorbic acids levels. Fish fed diets containing 1000 $mgkg^{-1}$ vitamin C exhibited comparatively higher lipid content ($P<0.05$). Although, hepatosomatic index (HSI) did not display any significant difference among treatments, VSI of experimental fingerlings was significantly affected by various ascorbic acids levels in the fed diet. Supplementation of vitamin C at 1000 mg kg^{-1}diet enhanced the growth performance and feed utilization of experimental fingerling of *B. sharpeyi*.

Keywords: *Barbus sharpeyi fingerling, Dietary vitamin C, Growth performance, Feed utilization, Carcass composition.*

1. Introduction

Vitamin C (ascorbic acid, AA) acts as a biological reducting agent for hydrogen transport. It is involved in many enzyme systems for hydroxylation, i.e., hydroxylation of tryptophan, tyrosine and proline. Vitamin C is necessary for optimum growth and maintenance (Dupee, 1966; Halver et al., 1969; Lovell, 1973; Mazik et al., 1987; Tewary and Patra, 2008). AA is an indispensable nutrient for fish, as they can not synthesize this nutrient due to the lack of enzyme L-gulonolactone oxidase (EC 1.1.3.8), which is responsible for synthesis of vitamin C de novo. Therefore, fish depend on exogenous supply of AA via an appropriate dietary source (Shiau and Lin, 2006). Inadequate supply of dietary vitamin C usually

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results in a number of deficiency signs such as reduced growth rate, spinal deformation, impaired collagen formation, internal haemorrhaging, increased mortality rate and abnormal pigmentation (Halver et al., 1969; Al-Amoudi et al., 1992; Gouillou-Coustans et al., 1998; Ai et al., 2004; Xie and Niu, 2006; Adewolu and Aro, 2009).

Benni (*Barbus sharpeyi* Güther, 1874), Actinopterygii, Cyprinidae, is an important rare native commercial fishery species in the Mesopotamian basin and highly valuable as freshwater food fish in the Karoon river region. Farm gate price for benni has been fluctuating between 2-3 USD kg – 1 in the past decade, and feed cost accounts for 70% of total production cost of this omnivorous species (De Silva and Anderson, 1995). Therefore, productivity and economical viability of commercial benni culture operations are highly dependent upon feed management.

The quantitative requirements for vitamin C have been determined for several species and there are considerable differences both between and within species in terms of proposed requirement by various studies (NRC, 1993). This discrepancy is probably related to fish species, fish size, and the differences in diet formulation and culture system (Ai et al., 2006). Although, a few data suggest that the use of dietary vitamin C in Cyprinidae improves their growth performance and feed utilization, the quantity is not well established. In the benni, in particular, the results are too scanty. Therefore, the aim of this study was to establish the effect of dietary vitamin C in common fish diets on the benni (*B. sharpeyi*) fingerling growth and feeding parameters, carcass composition and survival rate, paying special consideration to the quantity of the growth improvement.

2. Materials and Methods

2.1. Experimental System

The experiment was conducted in 15 polyethylene tanks (300 L) in Freshwater Fisheries Research Center, Ahwaz, Iran. Each tank was filled with freshwater up to 50 cm level which was maintained throughout the experimental period of 56 days. About 370 healthy benni fingerling were acclimatized in tanks for 2 weeks to prevail experimental condition of water temperature (25-27 °C) and pH (7.06 –7.61). Except during feeding, aeration was provided continuously to maintain dissolved oxygen above 6 mgl^{-1}. During the acclimation period, the fingerlings were fed on the basic diet without vitamin C (which later served as control diet) to satiation twice a day. After acclimation, 20 normal and healthy benni fingerlings were randomly selected to weigh and assigned to different tanks. Before weighing, fish were anesthetized using MS-222 (60 mg L^{-1}) to reduce stress. The average initial body weight and length of experimental fingerlings were 6.96±0.3 g and 7.5±0.33 cm, respectively. The water quality(pH, DO, Alkalinity, Ammonia) of the experimental tanks were monitored weekly following the methods of APHA (1992). During the experimental period, all fish were exposed to a natural photoperiod. Three replicates were maintained for each treatment.

2.2. Feeding Trial

The formulation of the basal diet (Hormoz Livestock Company, Iran) was such to provide 36.10% crude protein, 12.52% lipid, 11.21% ash and 33.21% carbohydrate and 6.87% moisture. Vitamin C (L-ascorbic acid, AA) was supplied by Hashtgerd Dietary Supplementation Company Limited, Iran. This was included at 0, 500, 1000, 1500 and 2000 mg kg^{-1} in the basic diet (AA was supplemented separately to the basal diet at the expense of wheat flour). The diets were prepared by thoroughly mixing the dry ingredients with oil then, adding cold water until it resulted in stiff dough. The dough was placed into a grinder for through mixing and extruded through 1.0 mm diameter strand. Diet was stored at -20 °C until use. During the experimental period, fish

were hand-fed at 3% body weight daily and two times a day (about 9 AM and 17PM) with corresponding experimental diets. Fish were weighed every two weeks and feed weight was adjusted accordingly. Uneaten food in each tank was collected 30 min after feeding by pipetting and then, dried at 75 °C. During the experimental period 25% of water in each tank was exchanged daily.

2.3. Proximate Analysis

Initial body composition of fish was analyzed using 15 samples of fish frozen at -20 °C prior to the commencement of trial. At the end of experiment, all fish were weighed. Three fish from each replicate were sacrificed for the proximate body composition analysis. In addition, liver and viscera of three fish from each tank were dissected and weighed to determine hepatosomatic index (HSI) and viscerosomatic index (VSI), respectively. Proximate composition of experimental diets and whole body proximate composition were analyzed using standard methods (AOAC, 1997). Each analysis was conducted in triplicate. Moisture was determined by drying the samples in an oven at 105 °C for 24 h to a constant weight; Ash was determined by incineration of samples in a muffle furnace at 550 °C for 12 h; Crude protein (N×6.25) was measured by Auto kjeldahl unit (Buchi, German; model B-414, K-438, K-371 and K-370); Total lipid was extracted from samples by homogenization in chloroform and methanol (2:1, v/v) (Bligh & Dyer, 1959), methylated and transesterified with boron trifluoride in methanol (AOAC, 1997).

2.4. Data Analysis

Growth performance was determined and feed utilization was calculated using the following formulas:

(1) Body weight gain (BWG, %) = [(final body weight (g) - initial body weight (g)) /initial body weight (g)] ×100.

(2) Specific growth rate (SGR, % day^{-1}) = [(Ln final weight - Ln initial weight) ×100]/ duration in days.

(3) Condition factor (CF) = (fish mass / fish total length3) ×100.

(4) Feed conversion ratio (FCR) = [dry weight of feed (g)/ wet weight gain (g)].

(5) Protein efficiency ratio (PER) = Increment in body weight (g)/ protein intake (g).

(6) Daily feed intake (FI, g d^{-1} $fish^{-1}$) = diet consumed × 100 / duration in days /fish number per tank

(7) Hepatosomatic index (HIS) = [weight of liver (g)/total weight of fish (g)] ×100.

(8) Viscerosomatic index (VSI) = [weight of viscera (g)/total weight of fish (g)] ×100.

(9) Survival rate (SR) = [final numbers of fish in each replicate / initial numbers of fish in each replicate] ×100.

Data were expressed as mean ± standard error. A one-way analysis of variance (ANOVA) was used to determine differences among supplementary levels, using SPSS 11.5 statistical software. Differences were considered significant at an alpha of 0.05 ($P<0.05$). Tukey's HSD multiple comparison test was used to determine significant differences among means.

3. Results

The growth performance and feed utilization of *B. sharpeyi* fingerlings fed with different levels of dietary supplementation of L-ascorbic acid are given in Table 1. Fish fed diet without ascorbic acid supplementation showed significantly lower growth ($P<0.05$) compared with those fed diets supplemented with various levels of ascorbic acids. The weight gain significantly increased linearly (3.85 g) with enhanced feeding supplementation levels up to 1000 mg kg^{-1} diet (Table 1), but no further increase was observed when feeding supplementation levels was increased beyond 1000 mg kg^{-1}. Body weight gain (BWG) and specific growth rate (SGR) followed a trend similar to

that of weight gain. BWG and SGR were significantly higher (P<0.05) in fish fed diet 2,whereas the control diet produced the lowest BWG and SGR. Condition factor (CF) was significantly (P<0.05) higher in fish in treatment 2 than those in treatments 1 and 4, but it did not differ from fish in treatment 3. Lower CF was observed in the control treatment. Variations in feed utilization did not follow similar pattern to that in the growth performances. Feed conversion ratio (FCR) was significantly highest (P<0.05) in fish fed control diet. FCR increased generally (P>0.05) with the increase in dietary vitamin C levels. There were significant differences in the protein efficiency ratio (PER) of fish fed on diets containing lower concentrations of ascorbic acids (0 and 500 mg kg^{-1}) compared with fish fed on diets containing higher doses of ascorbic acid (1000, 1500 and 2000 mg kg^{-1}) showing significantly (P<0.05) higher values in the later treatments. Fish fed control diet showed significantly lower percentage of survival rate (P<0.05) compared with those fed diets containing vitamin C supplements. Results showed that survival rate (%) did not display any significant difference among the diets supplemented with various levels of ascorbic acids.

Proximate composition (% wet weight) of carcass, hepatosomatic index (HSI) and viscerosomatic index (VSI) of *B. sharpeyi* fingerlings fed different levels of dietary supplementation of L-ascorbic acid are presented in Table 2. Vitamin C supplementation significantly (P<0.05) affected whole fish body composition except for moisture and ash. The body protein content increased significantly (P<0.05) with the increase in dietary ascorbic acids levels. Generally, ascorbic acids supplementation appeared to improved protein content. Fish fed the control diet had the lowest body protein content, while fish fed diets containing 1000 g AA kg^{-1} exhibited higher lipid content (P<0.05, the fish fed control diet produced the lowest lipid content). HSI did not display any significant difference among the treatments. Whereas, VSI of *B. sharpeyi* fingerlings was significantly (P<0.05) affected by various ascorbic acids levels, with fish fed diet containing 1500 g AA kg^{-1} diet showed significantly higher VSI as compared to the other treatments. There was no significant difference in VSI of experimental fish fed diet containing 1500 and 1000 g AA kg^{-1} diet (Table 2).

Table 1. Growth performance and feed utilization of *B. sharpeyi* fingerlings fed different levels of dietary supplementation of L-ascorbic acid.

Parameters	Treatment[a]				
	Control	Diet 1	Diet 2	Diet 3	Diet 4
Initial Weight (g)	6.90±0.32	6.96±0.26	7.03±0.30	7.06±0.27	6.89±0.34
Final Weight (g)	9.27±0.31^{c}	9.60±029 ^{b}	10.88±0.35 ^{a}	10.72±0.28 ^{a}	9.47±0.28 ^{bc}
Weight gain (g)	2.37±0.13 ^{c}	2.64±0.16 ^{b}	3.85±0.18 ^{a}	3.66±0.17 ^{a}	2.58±0.14 ^{bc}
BWG ^{b} (%)	34.35±2.26 ^{c}	37.93±1.98 ^{b}	54.77±2.14 ^{a}	51.84±1.77 ^{ab}	37.45±1.97 ^{b}
SGR ^{b} (% day^{-1})	0.53±0.04 ^{c}	0.57±0.03 ^{b}	0.79±0.01 ^{a}	0.74±0.02 ^{ab}	0.57±0.02 ^{b}
CF ^{b}	1.03±0.04 ^{b}	1.02±0.01 ^{b}	1.11±0.01 ^{a}	1.07±0.03 ^{ab}	1.05±0.02 ^{b}
FCR ^{b}	2.82±0.13 ^{a}	2.41±0.27 ^{b}	2.40±0.18 ^{b}	2.11±0.20 ^{b}	1.96±0.23 ^{b}
PER ^{b}	0.72±0.11 ^{c}	0.81±0.12 ^{b}	1.02±0.11 ^{a}	1.06±0.10 ^{a}	1.11±0.09 ^{a}
Survival rate (%)	91.6±2.8 ^{b}	96.6±2.1 ^{a}	96.6±2.7 ^{a}	96.6±2.4 ^{a}	98.3±2.1 ^{a}

Mean ± SE values (n=3) with different superscripts in each row are significantly different (P<0.05).
[a] Control, Diet 1, Diet 2, Diet 3 and Diet 4 supplemented with 0, 500, 1000, 1500 and 2000 mg L-ascorbic acid kg^{-1} diet, respectively.
[b] BWG (Body weight gain), SGR(Specific growth rate), CF (Condition factor), FCR (Feed conversion ratio), PER (Protein efficiency ratio).

Table 2. Proximate composition (% wet weight) of carcass, hepatosomatic index (HSI) and viscerosomatic index (VSI) of *B. sharpeyi* fingerlings fed different levels of dietary supplementation of L-ascorbic acid.

		Treatment[a]				
Parameters	**Initial**	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Moisture	75.93±0.5	74.82±0.7	73.32±0.7	73.50±0.8	72.98±0.7	73.87±0.7
Crude protein	57.45±0.28 ^{c}	61.19±0.25 ^{b}	61.20±0.14 ^{b}	61.27±0.21 ^{b}	61.51±0.21 ^{ab}	62.8±0.14 ^{a}
Crude lipid	28.44±0.21 ^{a}	21.11±0.24 ^{d}	21.67±0.18 ^{c}	22.31±0.21 ^{b}	21.34±0.25 ^{cd}	21.61±0.19 ^{c}
Ash	11.3±0.34 ^{b}	14.2±0.17 ^{a}	13.8±0.26 ^{a}	13.76±0.25 ^{a}	13.23±0.26 ^{a}	13.93±0.28 ^{a}
HIS (%)	1.02±0.12 ^{b}	1.31±0.10 ^{a}	1.33±0.09 ^{a}	1.36±0.09 ^{a}	1.33±0.10 ^{a}	1.26±0.11 ^{a}
VSI (%)	5.04±0.14 ^{c}	5.16±0.11 ^{b}	5.26±0.15 ^{b}	5.46±0.14 ^{a}	5.50±0.15 ^{a}	5.22±0.17 ^{b}

Mean ± SE values (n=3) with different superscripts in each row are significantly different (P<0.05).
[a] Control, Diet 1, Diet 2, Diet 3 and Diet 4 supplemented with 0, 500, 1000, 1500 and 2000 mg L-ascorbic acid kg^{-1} diet, respectively.

4. Discussion

Fish feed in this study included vitamin C, which is thought to be useful for normal growth of fish. The reduction in growth performance of fish fed the control diet in this study seems to indicate that AA has a specific effect on growth.The growth performance, feed utilization and survival of *B. sharpeyi* improved with the inclusion of vitamin C in fish diets. The weight gain, SGR and CF of benni fingerlings improved significantly with increasing supplementation of dietary ascorbic acid, and its growth peaked at between 1000 to 1500 mg AA kg^{-1} diet beyond which a decline was observed. The decline might probably be due to hypervitaminosis. Generally, the diet supplemented with 1000 mg AA kg^{-1} gave the best growth performance compared with the other diets. These results further confirmed that *B. sharpeyi* fingerlings needed adequate exogenous vitamin C to maintain normal growth and physiological functions, a finding agreeing well with previous studies on other fish (Al-Amoudi et al., 1992; Gouillou-Coustans et al., 1998; Shiau and Hsu, 1999; Wang et al., 2003; Mitra and Mukhopadhyay, 2003; Gbadamosi et al., 2006; Ibiyo et al., 2007). Tewary and Patra (2008) reported that among the four supplemented diets with vitamin C at 0, 500, 1000 and 1500 mg kg^{-1} tested on *Labeo rohita* fingerling, maximum growth was observed for fish fed diet supplemented with 1000 mg AA kg^{-1} while the lowest growth was observed for fish fed control diet. Alam et al. (2009) reported a similar result in Heteropneustes fossilis. Despite that, Ai et al. (2006) and Adewolu and Aro (2009) revealed that growth performances were not significantly influenced by different dietary supplementation of ascorbic acid levels in *Pseudosciaena crocea* and *Clarias gariepinus*, respectively. The different results reported is probably due to differences in fish species, size, the form of vitamin C and supplementation levels and experimental conditions in different studies. The minimum requirement for maximum growth was estimated to be 1000 mg ascorbic acid kg^{-1} diet in the present study. This value was higher than those reported for some fish species fed diets with stable forms of vitamin C (He and Lawrence, 1992; NRC, 1993; Thompson et al., 1993; Ai et al., 2004, 2006). It is generally believed that the metabolic rate is the primary factor regulating ascorbic acid requirements (Dabrowski, 1991) and growth rate always correlates with metabolic rate (Jobling, 1985; Ai et al., 2006). However, vitamin C requirement varies in different fish species. On the other hand, utilizing of different forms of dietary vitamin C is different in fish (Dabrowski et al., 1994; Ai et al., 2004).

In present research, the fish fed diets containing higher levels of vitamin C (1000-2000 mg kg^{-1}) showed significant (P<0.05) increase in the protein

efficiency ratio compared with fish fed diets containing lower levels of vitamin C (0 and500 mg kg^{-1}). The present results suggested that fish usually showed a requirement of nutrient to maximize body concentration, which was higher than that required for maximum growth. The acquiring of vitamin C always did not agree well with each other when different indices were used for evaluation (Ai et al., 2006). The results were similar to those reported by Alam et al. (2009) who found that FCR and PER improved linearly with increased ascorbic acid levels. Similarly, Tewary and Patra (2008) stated that PER significantly increased with enhanced feeding supplementation levels up to 1000 mg kg^{-1} diet, but no further increase was observed when feeding supplementation levels were increased beyond 1000 mg kg^{-1}. On the contrary, *C. gariepinus* fingerlings given the diet without vitamin C supplementation had significantly higher FCR and lower PER compared with those fed diets containing vitamin C supplements (Adewolu and Aro, 2009). It seems that the high concentration of vitamin C in diet 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; Tewary and Patra, 2008). In the present study, survival rate generally increased from 91.6% to 98.3% with increasing dietary vitamin C. When dietary vitamin C exceeded 500 mg kg^{-1}, the survival rate increased, suggesting that vitamin C was essential to maintaining the normal physiological function and survival for benni. Ibiyo et al. (2007) reported that survival of the fish increased significantly with dietary vitamin C inclusion and there was no significant difference between all the groups that received the vitamin C supplemented diets with respect to survival in *Heterobranchus longifilis* fingerlings. Similar results of dietary supplementation with vitamin C on percentage survival rate have been reported in *C. gariepinus* (Adewolu and Aro, 2009) and *Lateolabrax japonicus* (Ai et al., 2004).

According to the results of the present research, there were significant differences in final carcass composition among treatments especially with respect to crude protein and lipid content. From the results, it was clearly revealed that the protein content increased and fat contents fluctuated at the time of rearing and feeding trial. The significant improvement of whole body crude protein composition of the fish was an indication of the importance of vitamin C in body protein metabolism. Such improvement was also observed in *Oreochromis niloticus* (Soliman et al., 1994) and H. longifilis (Ibiyo et al., 2007). Vitamin C is an essential coenzyme in certain oxidative processes, including the oxidation of tyrosine and phenylalanine (Brander and Pugh, 1977; Ibiyo et al., 2007). However, Adewolu and Aro (2009) reported that the percentage of crude protein was not affected by dietary vitamin C treatments. Fish fed diet without vitamin C supplementation had the lowest percentage of body lipid compared with fish fed on other diets with vitamin C supplements. Correlation of growth performances with lipid content in benni fingerling in different groups demonstrate the different growth rate and different anabolic activity due to feeding different concentrations of vitamin C. The growth maintaining activity of ascorbic acid 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; Tewary and Patra, 2008). These results agree with previous studies on other species of fish (Ibiyo et al., 2007; Alam et al., 2009). Indices of condition, such as HSI and VSI, are often used to assess the nutritional status of fish because they can be determined easily and quickly and may provide an indication of physiological condition (Cui and Wootton, 1988; Desai and Singh, 2009). HSI were not significantly influenced ($P>0.05$) by supplementation vitamin C levels. Significant effect of dietary AA content was observed on VSI. Ibiyo et al. (2007) reported a similar result in H. longifilis.

Benni, the species used in this study, is one of the

important Cyprinidae used in extensive and semi-intensive fish farming throughout Tigris-Euphrates basin. The current study showed the positive effect of dietary vitamin C on feed utilization efficiency, body composition and growth performance of *B. sharpeyi* fingerlings during the experimental period. The results obtained indicated that successful aquaculture operations of this species could be enhanced through supplementation of the vitamin C level at 1000 mg kg^{-1} of feed. Results of this study could also serve as a working tool for the practical fish farmers in Iran who take benni as favorite species with considerable potential for aquaculture in many areas.

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