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Effect of Dietary Vitamin C Supplementation on the Blood Parameters of Mekong Giant Catfish (*Pangasianodon gigas*)

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ABSTRACT

The objective of this research was to determine the effects of vitamin C supplementation in feed on the hematology parameters of Mekong Giant Catfish (*Pangasianodon gigas* Chevey). Catfish with mean initial weight of 65 ± 2.00 g were randomly placed in 2.4 × 3 × 1.5 m cages in earthen pond. Growth experiments were conducted for 28 weeks. Four levels of vitamin C with three replicates each were tested including 0, 250, 500 and 750 mg kg^{-1} of feed. Glucose and total protein in serum were measured. Hematocrit, red blood cells, white blood cell count, and lysozyme activity of serum were examined. Fish received vitamin C supplemented diets gave higher weight gain. Catfish fed with vitamin C supplementary diets showed greater innate immunity response of Mekong giant catfish. The catfish received 500 and 750 mg vitamin C kg^{-1} supplementary diets showed the best records of hematocrit, red blood cell count, serum lysozyme, serum protein and serum glucose. These were significantly different from fish fed a basal feed with no additional vitamin C. But the increased in cost with no gain in performances was observed in catfish fed with 750 mg vitamin C kg^{-1} supplementary diet over those fed with the other levels of vitamin C tested. It was concluded that a minimum amount of 250 mg vitamin C kg^{-1} is sufficient to be added in diet for good growth performances and immune response of Mekong giant catfish.

Key Words: Vitamin C; Blood parameters; Mekong giant catfish; *Pangasianodon gigas*

INTRODUCTION

Mekong giant catfish (*Pangasianodon gigas*) is the world largest freshwater catfish that can grow up to 3 m in length with almost 300 kg in weight (Roberts & Vidthayanon, 1991). It is an endemic species of the main channel and tributaries of the Mekong River of Thailand, China, Laos PDR, Vietnam and Cambodia (Berra, 2001). It is classified as economically important fish species that has great commercial demands. These fish have been heavily caught from their natural habitats over many years that making this species a critically endangered fish (IUCN, 2005). This catfish has been bred successfully in ponds since 1983 (Phukasawan *et al*., 1984). However, this induced bred fish did not show its full growth potential as it seldom increased the weight of 5 kg/year in ponds (Polprasit & Tevaratmaneekul, 1997). Although marketable size of this catfish is not quite clear, customers prefer to taste the big ones since they familiar with eating the huge captured wild catfish. The slow growth of catfish in ponds is possibly due to feeding this fish with nutritionally unbalanced diets. Therefore, it is important to enrich the diets with vitamin C to enhance growth performance, survival rate and immunity.

Vitamin C (ascorbic acid) has been used in feeds for improving fish immunity and growth (Wang *et al*., 2003; Zhou *et al*., 2003). It also works as a cofactor in the hydroxylation of proline residues of connective tissue and collagen of vertebrates. Fish cannot synthesize vitamin C, because they do not have the L-gulonolactone oxidase enzyme, which is required to convert L-gulonic acid to ascorbic acid (Sato *et al*., 1976). For this reason, it is needed to add vitamin C in fish feed. In addition, vitamin C is not stable and usually degraded during feed processing and storage because of the exposure to light, moisture, oxygen, and high temperature.

Vitamin C deficiency has been shown to produce various abnormal signs in various fish including slow growth rate (Gouillou-Coustans *et al*., 1998), lordosis and scoliosis in rainbow trout (Hilton *et al*., 1978), impaired wound healing (Wahli *et al*., 2003), increased susceptibility to bacterial diseases (Ai *et al*., 2006), lower survival rates (Wang *et al*., 2003; Ai *et al*., 2006).

The vitamin C requirement varies with fish age, size, species, and rearing conditions. These requirements of vitamin C supplemented feed have been investigated in several fish species, and found that fish requirements for vitamin C ranged between 20 and 50 mg kg^{-1} feed (NRC, 1993). However, no quantitative information about vitamin C requirement for Mekong Giant Catfish is available. The objective of this study was to investigate the effects of vitamin C supplementation of feed on growth performance, blood parameters, and immunological modulation in Mekong giant catfish.

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MATERIALS AND METHODS

Catfish: Mekong Giant Catfish (MGC), *Pangasianodon gigas* of 60 ± 0.01 g body weight were brought to the laboratory from a local fish farm in Chiangmai. Fish were acclimated in a pond for 15 days and trained to accept the dry feeds.

Experimental design: The MGC with an initial weight of 65 ± 2.00 g were randomly divided into 12 groups of 30 fish each in 2.4 × 3 × 1.5 m cages installed in an earthen pond. This experiment consisted of 4 treatments: commercial pellet feed (30% crude protein, 7% crude fat, 5% crude fiber, 13% ash & 6% moisture) supplemented with 0, 250, 500, or 750 mg vitamin C kg^{-1} (vitamin C from L (+)-Ascorbic acid calcium salt dihydrate, Sigma, USA). Experimental pellet feeds were made by adding 300 mL of distilled water per kg of feed with or without vitamin C supplementation. After spraying, feeds were air-dried at 25°C for 6 h and kept in plastic bags at -4°C. Each treatment was carried out with three replicates. Catfish were fed to satiation twice a day for 28 weeks. The water quality parameters viz. temperature and dissolved oxygen (DO), pH and ammonia-nitrogen were measured according to the procedures by APHA, AWWA and WEA (1999).

Blood collection and analysis: Three catfish per replicate were randomly selected for blood samples taken from their caudal vein using heparinized syringes. White blood cell (WBC) and Red blood cell (RBC) were stained according to Natt and Herrick (1952). The hematocrit (Ht) values were obtained by a microhematocrit centrifugation technique. The serum was used for determining blood glucose with o-toluidine method (Hyvarinen & Nikkila, 1962; Winckers & Jacobs, 1971); Total protein was measured using Lowry reagent and read at absorbance at 620 nm on microplate reader (Lowry *et al*., 1951). The serum lysozyme activity was determined by the method of Puangkaew *et al*. (2004).

Statistical analyses: The data were presented as means±SD. The average values among treatments were analyzed by using one-way analysis of variance (ANOVA). When differences were found ($P < 0.05$), Tukey's test was applied to compare the means of different treatment with control groups.

RESULTS AND DISCUSSION

Sufficient vitamin C supplementation in fish feeds is necessary under intensive culture system for better survival and growth (Lin & Shiau, 2005) of tilapia (Al-Amoudi *et al.,* 1992), yellow croaker (Ai *et al*., 2006), parrot fish (Wang *et al.,* 2003), soft-shelled turtles (Zhou *et al*., 2003), and other economically important species. The importance of vitamins C supplementation in fish feed for growth improvement in Mekong giant catfish was clearly observed in this study. After the 7-month of feeding trial, giant catfish showed significant changes in their growth (Table I). A 250 mg kg^{-1} vitamin C in fish diet showed significantly better growth and feed conversion ratio in Mekong giant catfish. However, gilthead seabream received the vitamin C supplemented feed did not perform a statistically significant growth rate than one fed a vitamin C non-supplemented feed (Henrique *et al.,* 1998; Ortuño *et al*., 1999).

Aquatic environment especially non-suitable water parameters and high stocking density can act as a chronic stressor to catfish causing slow growth and suppress immunity. Maintenance of fish under good water conditions throughout rearing period is necessary for aquaculture business as its impairment can cause sudden death or serious disease outbreaks. Water quality characteristics of pond are presented in Table II. Water quality parameters viz. pH, DO, temperature, ammonia, and nitrites were not significantly different among treatments. However, water quality parameters were within the optimal values for fish production systems.

Values for red blood cell count (RBC), white blood cell count (WBC) hematocrit (Ht), serum proteins and glucose of catfish fed with the experimental diets are presented in Table III. There were non significant differences in Ht values among treatments except for 50 mg vitamin C kg^{-1} supplementation. However, a significant increase in the catfish RBC number under the treatment of 750 mg vitamin C kg^{-1} was observed. Similar results were observed in pirarucu (*Arapaima gigas*) received 800 and 1200 mg vitamin C kg^{-1} supplementary diets (Andrade *et al.,* 2007). Vitamin C is a powerful antioxidant protecting against oxidative damage to various tissues of fish including red blood cells (Sahoo & Mukherjee, 2003). Therefore, Ht and RBC can act as oxidative status indicators since erythrocytes are one of the main production sites of free radicals. Kiron *et al*. (2004) indicated that some of these free radicals are able to trigger peroxidation of unsaturated fatty acids in their phospholipid membranes, leading to changing in the erythrocyte size, number and their integrity. Moreover, Adham *et al*. (2000) demonstrated that vitamin C insufficient feeds cause macrocytic anemia, characterized by a decrease in the hemoglobin, reduction in number of erythrocytes and hematocrit.

Plasma glucose is one of the stress indicators in fish (Menezes *et al*., 2006). However, differences in physiological status of fish possibly result in the variation of glucose concentration in fish serum. An increase in serum glucose was noticed in *Pangasianodon gigas* fed with diets containing 750 mg vitamin C kg^{-1} (Table III). On the other hand, plasma glucose has been reported significantly higher in seabream, *Sparus aurata* received no vitamin C supplemented feed and exposed to hypoxic stress in relation to other groups (Henrique *et al*., 1998).

High vitamins C in fish feed could enhance protein synthesis (Andrade *et al*., 2007), Total serum protein in Mekong giant catfish fed high vitamin C concentrations slightly increased after 8 weeks of experiment (Table III). Similar result was found in *Arapaima gigas* by Andrade *et al*. (2007). Diets with high vitamin C levels also enhanced

Table I: Effects of vitamin C supplementary diets on growth performance after 7 month culture

Characters	Vitamin C levels in experimental feeds (mg/kg)				
	0	250	500	750	P-value
Average initial weight (g/fish)	68.11 ± 2.11	65.00 ± 2.34	66.56 ± 2.22	67.56 ± 1.02	0.310
Final weight (g/fish)	294.00 ± 11.46 [a]	337.78 ± 17.53 [b]	347.44 ± 5.54 [b]	334.00 ± 18.10 [b]	0.008
Weight gain (g/fish)	225.89 ± 11.97 [a]	272.78 ± 18.46 [b]	280.89 ± 5.55 [b]	266.45 ± 19.00 [b]	0.008
Average initial length (cm)	22.89 ± 0.54	21.95 ± 1.21	22.06 ± 0.41	22.01 ± 0.84	0.479
Average final length (cm)	32.90 ± 0.34 [a]	33.43 ± 0.44 [ab]	34.88 ± 1.16 [b]	34.57 ± 0.44 [ab]	0.023
Increased length (cm)	10.00 ± 0.24	11.48 ± 1.17	12.82 ± 1.51	12.55 ± 1.26	0.061
Specific growth rate (%/day)	0.69 ± 0.03 [a]	0.77 ± 0.03 [b]	0.77 ± 0.02 [b]	0.75 ± 0.03 [ab]	0.015
Survival rate (%)	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	-
Feed Conversion Ratio (FCR)	2.36 ± 0.29 [a]	1.58 ± 0.03 [b]	1.71 ± 0.09 [b]	1.69 ± 0.15 [b]	0.002
Fish production (g/cage)	6776.67 ± 359.07 [a]	8183.33 ± 553.74 [b]	8426.67 ± 166.53 [b]	7993.33 ± 570.12 [b]	0.008

*Different superscript letters in same row indicate significant differences at $P < 0.05$ (ANOVA)
No significant differences in parameters without superscript letter (Tukey's test $p<0.05$)

Table II: Water parameters in Mekong giant catfish culture under different treatments

Treatments	Temperature (°C)	pH	DO (mg L^{-1})	Ammonia (mg L^{-1})	Nitrite (mg L^{-1})
Control	25.73 ± 0.13	7.22 ± 0.10	4.84 ± 0.06	1.164 ± 0.283	0.132 ± 0.067
C250	25.67 ± 0.13	7.20 ± 0.03	4.77 ± 0.15	0.875 ± 0.290	0.052 ± 0.008
C500	25.63 ± 0.19	7.18 ± 0.04	4.75 ± 0.12	1.112 ± 0.224	0.053 ± 0.009
C750	25.71 ± 0.08	7.19 ± 0.04	4.73 ± 0.18	1.026 ± 0.123	0.078 ± 0.026
(P - value)	0.775	0.832	0.749	0.511	0.085

Control=commercial diet; C250, C500, and C750 were 250, 500, and 750 mg vitamin C kg^{-1} supplementary diets, respectively
The data are shown in Mean ± S.D
No significant differences in any of the parameters were found (Tukey's test $p<0.05$)

Table III: Hematocrit, Red blood cell count, White blood cell count, Serum glucose, Serum protein, and lysozyme activity of Mekong Giant Catfish fed with different vitamin enriched diets for 28 weeks

Characters	Vitamin C levels in experimental feeds (mg/kg)				
	0	250	500	750	P-value
Hematocrit (%)	35.25 ± 0.34 [a]	33.91 ± 0.22 [b]	35.11 ± 0.55 [a]	34.70 ± 0.49 [ab]	0.018
Red blood cell (× 10^6 cell/mm^3)	2.35 ± 0.06 [a]	2.49 ± 0.00 [ab]	2.47 ± 0.09 [ab]	2.64 ± 0.06 [b]	0.004
White blood cell (× 10^4 cell/mm^3)	10.17 ± 0.34	9.42 ± 0.36	9.62 ± 0.60	9.70 ± 0.68	0.391
Serum lysozyme (unit/min)	2.82 ± 0.73	2.89 ± 0.53	3.28 ± 0.49	3.06 ± 0.36	0.737
Serum protein (g/dL)	3.81 ± 0.33	3.98 ± 0.24	3.75 ± 0.45	4.02 ± 0.03	0.653
Serum glucose (mg/dL)	0.68 ± 0.03 [a]	0.72 ± 0.02 [ab]	0.72 ± 0.03 [ab]	0.75 ± 0.03 [b]	0.050

*Different letters in same row indicate significant differences at $P < 0.05$ (ANOVA)
No significant differences in parameters without superscript letter (Tukey's test $p<0.05$)

the immune responses and increased the O_2 carrying capacity in pirarucu (Andrade *et al*., 2007). In contrast, total serum protein was not found to be affected by dietary ascorbate levels in juvenile turbot, *Scophthalmus maximus*. The total protein in serum can be used as an indicator of fish innate immune responses (Ortuño *et al*., 1999; Sahoo & Mukherjee, 2002; Ai *et al*., 2004; Lin & Shiau, 2005) due to the enhanced activity of lysozymes (Roberts *et al*., 1995; Cuesta *et al*., 2002; Ai *et al*., 2004; Lin & Shiau, 2005; Eo & Lee, 2008).

Lysozyme is liberated by leukocytes and plays a crucial role in antimicroorganism activity. It is an enzyme attacking the β-1, 4 glycosidic bond in the peptidoglycan of bacterial cell walls and lyse some Gram positive bacteria. Alexander and Ingram (1992) stated that this enzyme works in conjunction with complement targeting to Gram-negative bacteria. In these findings, lysozyme activities were greater in catfish received feeds with 250 mg vitamin C kg^{-1} or higher. This vitamin improved the lysozyme activity also found in yellow croaker, *Pseudosciaena crocea* (Ai *et al*., 2006), juvenile turbot, *Scophthalmus maximus* (Lin & Shiau, 2005), Japanese seabass, *Lateolabrax japonicus* (Ai *et al*., 2004), tiger puffer, *Takifugu rubripes* (Eo & Lee, 2008).

Fish received higher vitamin C concentrations presented better immunological responses when exposed to chronic stress including crowding (Montero *et al.,* 1999) and hypoxia (Henrique *et al*., 1998). Large yellow croaker fed with high dietary vitamin C diets increased more resistance to *Vibrio harveyi* infection (Ai *et al*., 2006). The fish pathogen resistance improvement were observed in other fish; for example, resistance to *Aeromonas hydrophila* was found in mrigal (Sobhana *et al*., 2002); resistance to *A. salmonicida* in Atlantic salmon (Hardie *et al*., 1991) and resistance to *V. anguillarum* in rainbow trout (Navarre & Halver, 1989). However, channel catfish, *Ictalurus punctatus*, fed with elevated vitamin C supplementary diets did not show higher resistance to *Edwardsiella ictaluri* infection (Li *et al.,* 1993).

In intensive culture system with high stocking density and frequently fluctuating weather, vitamin C is required to be added into fish diets. To improve fish immune responses, feeding fish with much higher dietary vitamin C concentrations are necessary for fish growth (Henrique *et al*., 1998; Ortuño *et al*., 2003). A tiger puffer needs exogenous vitamin C and the suitable level could be 29 mg vitamin C kg^{-1} feed for normal physiology and growth while over 82 mg vitamin C kg^{-1} is required to improve innate immune responses (Eo & Lee, 2008). However, an overdose of dietary ascorbic acid (>160 mg kg^{-1}) did not show better innate immune responses. As feed supplemented with 750 mg vitamin C kg^{-1} increased the cost with no effect on fish growth. In addition, the combination of elevated doses of vitamin C and other immunostimulants including vitamin E, glucan, bovine lactoferrin, herbs and oligosaccharides from yeast had a positive impact on both the non-specific and specific immune parameters in fish (Chitmanat, 2002).

In conclusion, the high vitamin C concentrations are able to enhance growth performance and immune responses of Mekong giant catfish that can protect them under unforvable conditions. These conditions should be further elucidated using deadly infectious pathogens or cold stress, considering the economic feasibility of vitamin C application in fish feeds at an appropriate time as well.

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