

Performance and hematology of pacu fed diets supplemented with vitamins C and/or E

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ABSTRACT: Pacu (*Piaractus mesopotamicus* Holmberg, 1887) is a valued Brazilian fish species for aquaculture. This is highly susceptible to disease, and feed supplementations for pacu can be a very important strategy to prevent disease incidence in fish farms. The aim of this study was to evaluate a strategic supplementation for pacu. Juvenile pacu (10.5 ± 1.2 g) were fed diets containing three levels of vitamins C and/or E (0, 250, and 500 mg vitamin kg⁻¹ diet). Fish were fed diets without supplementation for two months prior to the experiment. After that period, experimental feeding was initiated for two months. Growth and hematological evaluations were made on the thirtieth and sixtieth days of feeding. Pacu fed diet without supplementation of vitamins C and E during 120 days did not show clear typical signs of deficiency. Fish fed diet vitamins C and E free increased feed intake, but no improvement on growth performance was detected. Vitamin E proved essential for erythrocyte protection, so that the higher the level of this vitamin in diet, the smaller the number of erythroblasts. Supplementation with 500 mg of vitamin C and 250 mg of vitamin E for 60 days increased the production of monocytes, thrombocytes and special granulocytic cells in pacu.

Key words: *Piaractus mesopotamicus*, ascorbic acid, feed supplementation, tocopherol

Desempenho e hematologia do pacu alimentado com dietas suplementadas com vitaminas C e/ou E

RESUMO: O pacu (*Piaractus mesopotamicus* Holmberg 1887) é uma espécie altamente valiosa para a aqüicultura brasileira. Entretanto, trata-se de uma das espécies mais susceptíveis a doenças. Por isso, a suplementação alimentar para o pacu pode representar importante estratégia de prevenção da incidência das enfermidades em pisciculturas. Pacus (10,5 ± 1,2 g) foram alimentados com dietas contendo três níveis de vitamina C e/ou E (0, 250 e 500 mg vitamina kg⁻¹ de dieta). Os peixes foram alimentados com dieta não purificada sem suplementação durante os primeiros 60 dias para reduzir as reservas teciduais das vitaminas. Após este período, os peixes foram alimentados com as dietas testadas durante 60 dias. As avaliações de crescimento e hematologia foram realizadas no 30º e 60º dias. Pacus alimentados com dieta sem suplementação com as vitaminas C e E durante 120 dias não apresentaram sinais claros típicos de deficiência. Os peixes que receberam a dieta deficiente em vitamina C e E apresentaram maior consumo de ração, porém sem melhora no desempenho produtivo. A vitamina E mostrou-se essencial para a proteção dos eritrócitos, sendo que quanto maior o nível desta vitamina na dieta, menor o número de eritroblastos. A suplementação com 500 mg de vitamina C e 250 mg de vitamina E durante 60 dias aumentou a produção de monócitos, trombócitos e células granulocíticas especiais em pacu.

Palavras-chave: *Piaractus mesopotamicus*, ácido ascórbico, suplementação alimentar, tocoferol

Introduction

Pacu (*Piaractus mesopotamicus* Holmberg 1887) is highly valued Brazilian fish species for aquaculture due its acceptance by consumers and high growth rate. Its diet includes leaves, plant residues, fruit, which are rich in vitamins (Abimorad et al., 2009; Abimorad and Carneiro, 2007; Jomori et al., 2003). In fish farms, vitamins C and E should be antioxidant compounds of great value for this species, which is highly susceptible to diseases under stressful conditions (Martins et al., 2002).

Vitamin C contributes toward both bone and cartilaginous tissue formation and its deficiency in the diet causes bone and gill deformations, body and internal-organ hem-

orrhages, lack of appetite and an increase in the negative consequences of stress (Mitoma and Smith, 1960). This vitamin in diets for aquatic organisms prevents the negative effects of stress and enhances their immune response (Petric et al., 2003; Sarma et al., 2009).

Vitamin E protects cell membranes against lipid peroxidation and its deficiency causes reduction on survival index, deformations in erythrocyte membranes, high *in vitro* hemolysis, loss of appetite, low feed conversion efficiency, muscular dystrophy and shortening of the opercula (Chien et al., 1999; Garcia et al., 2007; Hamre et al., 1997; Sau et al., 2004; Tocher et al., 2002).

Studies of hematological variables have become important as an auxiliary tool for the diagnosis and determination of fish health status (Tavares-Dias and Moraes, 2004). Con-

sequently, cells should be identified and quantified to clarify the role of these vitamins in pacu defense mechanisms and to indicate the nutrient requirements for this species to improve its performance and health. The aim of this investigation was to evaluate growth performance and hematological parameters of pacu fed diets supplemented with different levels of vitamins C and/or E and study their interactions to recommend a strategic supplementation for farmers.

Material and Methods

The experiment was carried out in a randomized block design with three blocks (replications), in a 3 × 3 factorial combination. Each block constituted of different condition under which tanks were placed, inside or outside of the laboratory. Three vitamin C and three vitamin E levels in feed (0, 250, and 500 mg kg⁻¹ of vitamin) were tested. Juvenile pacu (10.5 ± 1.2 g) were distributed in tanks (300 L) with water renewal and continuous aeration at a stocking density

of 14 fish/tank in Jaboticabal (21°14' S; 48°18' W), São Paulo State, Brazil.

To decrease fish vitamin reserves, fish were stocked during two months in an environment with low incidence of light, preventing occurrence of phytoplankton, which is rich in vitamin C. The diet offered in this period was also vitamin C and E free. After that, fish were fed experimental diets at a rate equal to 5% of the weight of the fish in two feedings daily for two months.

The basal diet was formulated and prepared to reach the nutritional requirements of pacu (Table 1). A vitamin and mineral supplement used did not contain vitamins C and E. The diet was prepared as follows: all ingredients were finely ground and weighed. The vitamin and mineral supplement, as well as the vitamin C and E sources at the desired levels, were incorporated into ground corn and the remaining ingredients. Feed was pelletized (65°C) using a die which produced pellets with 2.5 mm in diameter and 7 mm in length.

Table 1 – Composition of basal and experimental diets, expected and detected vitamin C and vitamin E levels added to the experimental diets.

Ingredient	%			
Soybean bran	26.22			
Corn	31.13			
Wheat bran	28.58			
Fish meal	11.62			
Soybean oil	1.95			
Vitamin and mineral supplement*	0.50			
Calculated Composition				
Crude Protein (%)	26.00			
Ether Extract (%)	7.00			
Crude Fiber (%)	5.81			
Gross Energy (kcal kg ⁻¹ feed)	4,150			
Nitrogen-free Extract (%)	44.00			
Mineral Matter	6.77			
Treatment	Vitamin C		Vitamin E	
	Expected Level		Detected Level	
----- mg kg ⁻¹ -----				
1	0	0	trace**	trace
2	250	0	234	trace
3	500	0	461	trace
4	0	250	trace	223
5	0	500	trace	460
6	250	250	233	251
7	250	500	240	482
8	500	250	482	235
9	500	500	484	519

*Vitamin and mineral supplement composition: vitamin A 1,200,000 IU, vitamin B1 4,800 mg, vitamin B12 4,800 mg, vitamin B2 4,800 mg, vitamin B6 4,800 mg, vitamin D3 200,000 IU, vitamin K3 2,400 mg, Folic acid 1,200 mg, Biotin 48 mg, Calcium pantothenate 12,000 mg, Choline chloride 108 g, Niacin 24,000 mg, Selenium 100 mg, Iodine 100 mg, Cobalt 10 mg, Copper 3,000 mg, Iron 50,000 mg, Manganese 20,000 mg, Zinc 30,000 mg, vehicle 1,000 g, antioxidant 25 g. **trace of vitamins.

Vitamin C and E sources used were ascorbyl polyphosphate – 35% activity and tocopherol adsorbate 50% activity, respectively. The vitamin concentration calculations for each treatment were made based on vitamin activity of the products. Feed was stored in plastic bags and frozen. Feed samples were analyzed to determine vitamins C and E levels in the diets (Table 1).

The following aquatic variables were monitored daily during the experimental period: temperature and dissolved oxygen, with an oximeter YSI model 55, pH, with a Corning meter and water conductivity, with a Corning conductivitymeter. Water renewal rate was controlled once a week. Aquatic variables were maintained within recommended values for fish welfare: temperature $30.5 \pm 1.8^\circ\text{C}$, dissolved oxygen $4.8 \pm 0.8 \text{ mg L}^{-1}$, electric conductivity $204.1 \pm 28.1 \mu\text{S cm}^{-1}$ and pH 8.6 ± 1.1 . Water renewal rate maintained at $45 \pm 15 \text{ mL s}^{-1}$.

Feed intake (FI) was determined once a week. Monthly, fish were measured and weighted to determinate weight gain (WG), feeding conversion (FC) and condition factor (CF), according the equations 1 to 3:

$$\text{WG (g)} = \text{Final weight} - \text{Initial weight} \quad (1)$$

$$\text{FC} = \text{FI/WG} \quad (2)$$

$$\text{CF} = [\text{Weight (Length)}^3] \times 100 \quad (3)$$

Blood was collected and analyzed monthly during the two months of feeding with experimental diets. Blood aliquots (1.0 mL) were collected by puncturing the anal fins of two specimens per tank in each collection, using syringes containing EDTA (10%). Erythrocyte counts were obtained with an automatic blood cell counter (Celm Model CC510) and the hemoglobin rate was obtained according to Collier's method (1944). Hematocrit was determined according to recommendations of Goldenfarb et al. (1971). Corpuscular constants such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Wintrobe (1934). Differential leukocyte counts were obtained by preparing panchromatically-stained smears. Cells were examined under light microscopy. Up to 200 cells per smear were counted, and percentages were established for each cell component of interest. Total leukocytes, total thrombocytes, and erythroblasts were indirectly quantified in the same smears by counting the numbers of thrombocytes, leukocytes, and erythroblasts for each 2,000 erythrocytes (Tavares-Dias and Moraes, 2004).

ANOVA assumptions were evaluated by the Shapiro-Wilk's tests for residue normality and Levene's test for homogeneity of variances. A $3 \times 3 \times 2$ factorial combination was used to analyze the influence of the main effects (three vitamin C levels, three vitamin E levels, and two evaluation times) and the interactions between them (vitamin C \times vitamin E, vitamin C \times time, and vitamin E \times time), using two-way factorial ANOVA (Steel and Torrie, 1960). Means that showed differences between the factors were compared by Tukey's test ($p = 0.05$).

Results and Discussion

Levels of vitamins C and E detected in the diet were similar to the levels added (Table 1). No typical signs of deficiency were observed in fish during the studied period. Scurvy signs in rainbow trout (*Onchorynchus mykiss*), such as scoliosis, lordosis, dark skin color, pale gills, lethargy and swimming on the tank surface appear after 18 weeks of feeding vitamin C free (Navarre and Halver, 1989). Pintado (*Pseudoplatystoma corruscans*) fed a vitamin C-deficient diet for 12 weeks showed head convexity, fragile fins, and a posteriorly-displaced mandible (Fujimoto and Carneiro, 2001). It is possible that carnivorous species, such as trout and pintado, are more susceptible to this vitamin deficiency and must have higher requirements for these vitamins than omnivorous species (Abimorad and Carneiro, 2007; Fujimoto and Carneiro 2001; Mitoma and Smith, 1960).

Supplementation with vitamins C and E changes fish feed intake: the greater the vitamin deficiency the greater the feed intake (Table 2). This behavior has not been observed in other fish species (Bai and Lee, 1998; Fujimoto and Carneiro, 2001; Halver, 1953). However, for pacu it is suggested that the species tries to supply these vitamin deficiencies by an intake of a greater amount of feed. In a natural habitat, this species has a peculiar behavior of looking for better food, varying the sources as a function of season. Wild pacu ingests diversified foods, including leaves, plant residues, fruit and much less often, fish and/or mollusks or crustaceans (Abimorad and Carneiro, 2007). Anyway, the improvement on feed intake did not increase performance parameters because of the damage caused by deficiency on fish growth.

No effect ($p > 0.05$) of vitamin supplementation was observed in this assay on weight gain, food conversion efficiency and feeding conversion. The influence of vitamins on fish performance has not been completely clarified. Similar results has been found in studies on vitamin C and E supplementation for channel catfish (*Ictalurus punctatus*), *Labeo rohita*, piauçu (*Leporinus obtusidens*), and golden shiner (*Notemnogon crysoleucas*) (Chen et al., 2004; Ortuño et al., 2001; Mello et al., 1999). On the other hand, many studies have reported the positive effect of vitamin supplementation. Tilapia hybrids, *Oreochromis niloticus* \times *Oreochromis*

Table 2 – Comparison between mean feed intake in *Piaractus mesopotamicus* fed diets supplemented with vitamin C and/or E.

Vitamin C	Vitamin E (g per day)		
	0	250	500
----- mg kg ⁻¹ -----			
0	59.48 a	54.75 ab	54.44 b
250	54.76	56.99	55.90
500	55.47	55.86	54.65

Note: Identical letters in rows or columns: no difference ($p > 0.05$). Lower case letters compare rows and upper case letters compare columns.

aureus fed diet supplemented with an adequate level of vitamin C but vitamin E free had smaller weight gain and feeding conversion than groups fed optimum levels of both vitamins. Shiau and Hsu (2002) suggested that vitamin C supplementation at high levels (three times higher than the adequate level) can make up for the lack of vitamin E in the diet. Supplementation with zero or 160 mg kg⁻¹ vitamin E in feed associated with 250 mg kg⁻¹ vitamin C over a 32-week period increased the growth rate of *Perca flavescens*. So, in the long run, supplementation with vitamins C and E may improve fish performance (Lee and Dabrowski, 2004).

Regardless of the vitamin level in the diet, feed intake and food conversion efficiency increased from the first to the second month ($p \leq 0.05$), while the condition factor was reduced. Erythrocyte, hematocrit, total protein, globulin, hemoglobin, MCHC, and thrombocyte counts increased from the first to the second month ($p \leq 0.01$), while there were reductions in MCV and MCH values ($p \leq 0.01$). Vitamin E affected the number of erythroblasts, so that the higher the level of this vitamin in the diet, the smaller the number of these cells (Figure 1). Erythropoiesis in fish occurs in the spleen and kidneys. Young erythrocyte forms are called erythroblasts, characterized by larger cell and nucleus sizes than mature erythrocytes (Tavares-Dias and Moraes, 2004). Vitamin E is a powerful antioxidant which extends erythrocyte life and is essential for cell respiration (Hung et al., 1981). Vitamin E deficiency induces erythrocyte fragility, causing their degeneration in many species such as Channel catfish (Wilson et al., 1984; Wise et al., 1993), Atlantic salmon (Hamre et al., 1997), rainbow trout (Furones et al., 1992), and pacu (Belo et al., 2005). Although erythrocyte fragility was not measured, fish fed a vitamin E-deficient diet showed lysed erythrocytes in blood smears. Therefore, to maintain an adequate erythrocyte level, it is suggested that fish should increase the production of erythroblasts to balance their loss in the blood.

Vitamin supplementation did not change hematocrit and hemoglobin values; however, these parameters were increased

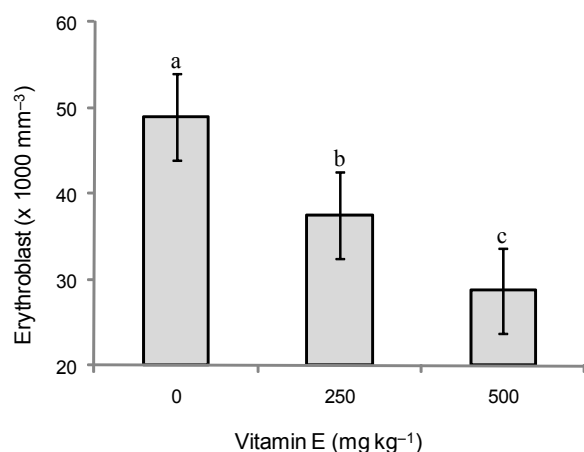


Figure 1 – Comparison between mean erythroblast counts in the blood of *Piaractus mesopotamicus* fed increasing levels of vitamin E during 60 days. Identical letters no difference ($p > 0.05$).

from the first to the second month, as observed by Chen et al. (2003 and 2004) in golden shiner. Tavares-Dias et al. (2000a, b) observed a positive correlation ($p \leq 0.01$) between those blood parameters. Significant interaction was observed between vitamins C and E on the number of special granulocytes. The highest value found for this cell type was observed in the group which received the diet without vitamin C supplementation (0 mg kg⁻¹) and 250 mg kg⁻¹ vitamin E (Table 3). There was also interference of vitamin E supplementation on the number of special granulocytes (Table 4). The highest value found for this cell type was observed in the group which received the diet supplemented with 250 mg kg⁻¹ vitamin E for 60 days.

Table 3 – Comparison between mean numbers of special granulocytes ($\times 1,000 \text{ mm}^{-3}$) of *Piaractus mesopotamicus* fed diets supplemented with vitamin C and/or E.

Vitamin C	Vitamin E		
	0	250	500
----- mg kg ⁻¹ -----			
0	0.23 b	2.36 La	0.47 b
250	0.45	0.39 B	0.21
500	0.54	0.61 B	0.68

Note: Identical letters in rows or columns: no difference ($p > 0.05$). Lower case letters compare rows and upper case letters compare columns.

Table 4 – Comparison between mean numbers of thrombocytes, monocytes, and special granulocytes (SG) of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E as a function of feeding time.

Vitamin C	Period	
	30 days	60 days
thrombocytes		
----- $\times 1,000 \text{ mm}^{-3}$ -----		
0	47.18	62.21 C
250	46.22 b	74.53 Ba
500	41.93 b	90.40 La
monocytes		
----- $\times 1,000 \text{ mm}^{-3}$ -----		
0	1.30 AB	1.33 B
250	1.71 A	1.32 B
500	1.05 Bb	2.40 La
special granulocytes		
----- $\times 1,000 \text{ mm}^{-3}$ -----		
0	0.42	0.39 B
250	0.50 b	1.74 La
500	0.38	0.52 B

Note: Identical letters in rows or columns: no difference ($p > 0.05$). Lower case letters compare rows and upper case letters compare columns.

At 60 days of feeding, the number of thrombocyte cells increased to the level of vitamin C in the diet. In fish fed the two higher vitamin levels in the diet (250 and 500 mg kg⁻¹), the number of thrombocytes increased from the first to the second month (Table 4). Effect of this vitamin on the improvement of the number of thrombocytes in golden shiner was already reported (Chen et al., 2004). Bozzo et al. (2007) and Tavares-Dias et al. (2007) suggested that thrombocytes, as well as leukocytes, also have hemostatic functions and act as defense cells in fish. Supplementation with vitamin C increased the number of those cells in pacu; after the challenge by *Aeromonas hydrophila*, fish which received higher vitamin C levels showed a reduction on circulating thrombocytes, suggesting a migration of these cells to inflammation sites (Garcia et al., 2007).

The number of monocytes increased from the first to the second month of feeding only in the group which received the highest level of vitamin C in the diet (500 mg kg⁻¹) (Table 4). In studies with the same species, feed supplementation with 500 mg kg⁻¹ feed of vitamin C increased macrophages accumulation, as well as the formation of macrophage polykaryons on glass coverslips implanted into the subcutaneous tissue of *P. mesopotamicus* (Petric et al., 2003).

P. mesopotamicus which received diet deficient in vitamins C and E increased the feed intake, but no improvement on growth performance index could be seen. Vitamin E was essential for erythrocyte protection, because fish which received 500 mg of this vitamin presented a reduction in the erythroblast number. Supplementation with 500 mg of vitamin C and 250 mg of vitamin E for 60 days increased the production of important cells of non specific defense mechanisms: monocytes, thrombocytes and special granulocytic cells in *P. mesopotamicus* and can be used as an important strategic management for pacu of fish farms.

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