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VITAMIN A SUPPLEMENTED DIET FOR PACU FINGERLINGS

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ABSTRACT

Vitamins are present in small amounts in food and are essential for the organic balance of fish. Liposoluble vitamins are stored in the liver and may lead to hypo or hypervitaminosis which in both cases can generate complications to animal homeostasis. This study aimed to evaluate the effects of vitamin A supplementation in the diet of pacu fingerlings (*Piaractus mesopotamicus*). A total of 240 fingerlings with the initial average weight of 17.55 ± 3.22 g were randomly distributed in 20 500 L-circular tanks. Diets were supplemented with retinol acetate (1,000,000 IU of vitamin A g^{-1}) to contain 0, 3,000, 6,000, 9,000, and 12,000 IU of vitamin A kg^{-1} of diet. The fish were fed *ad libitum*. Productive performance, carcass yield, visceral fat, hepatosomatic ratio, carcass chemical composition, hematology blood parameters, and liver histology were evaluated. The quadratic effect was observed on final weight and apparent feed conversion with optimum levels at 6,583 and 5,555 IU of vitamin A kg^{-1} of feed, respectively. There was no influence of vitamin A supplementation on survival, carcass chemical composition, hematology, and liver histology. The minimum supplementation of 5,555 IU of vitamin A kg^{-1} of ration is indicated to obtain enhanced results in weight gain and apparent feed conversion in pacu fingerlings.

Key words: blood parameters; histology; liposoluble vitamin; native fish; pisciculture.

VITAMINA A NA DIETA DE ALEVINOS DE PACU

RESUMO

As vitaminas estão presentes em pequenas quantidades nos alimentos e são essenciais para o equilíbrio orgânico dos peixes. As vitaminas lipossolúveis são armazenadas no fígado, podem apresentar sinais clínicos de hipovitaminose ou hipervitaminose, e que em ambos os casos podem trazer complicações a homeostase animal. O objetivo do presente trabalho foi avaliar a suplementação de vitamina A em dietas para alevinos de pacu (*Piaractus mesopotamicus*). Foram utilizados 240 alevinos com peso inicial médio de 17,55 ± 3,22, distribuídos aleatoriamente em 20 caixas circulares de 500 L. As dietas foram suplementadas com acetato de retinol (1.000,000 IU of vitamin A g^{-1}), de forma a conter 0, 3.000, 6.000, 9.000 e 12.000 UI de vitamina A/kg de dieta. Foi observado efeito quadrático sobre os parâmetros de ganho de peso e conversão alimentar aparente com níveis ótimos de 6.583 e 5.555 UI de vitamina A kg^{-1} de ração, respectivamente. Não foram observadas influencias da suplementação de vitamina A sobre sobrevivência, composição da carcaça, hematologia e histologia do fígado do pacu. Para obtenção de melhores resultados de ganho de peso e conversão alimentar indica-se a suplementação mínima de 5,555 UI de vitamina A kg^{-1} de ração para o pacu.

Palavras-chave: parâmetros sanguíneos; histologia; vitamina lipossolúvel; peixes nativos; piscicultura.

INTRODUCTION

Vitamins are organic compounds that are classified as liposoluble or hydrosoluble and function as catalysts or metabolism regulators. Liposoluble vitamins, including vitamin A, are absorbed through the small intestine along with lipids from the diet. Thus, conditions that are favorable for the absorption of fats provide an increased absorption of liposoluble vitamins (NRC, 2011).

Vitamin A participates in numerous functions in organisms and is involved in the synthesis of some glycoproteins and glycosaminoglycans, which act as steroid hormones in growth regulation and cell differentiation (HALVER, 2002; FURUITA *et al*., 2003). According to HALVER (2002), the vitamin requirements in fish vary with age, size,

environmental factors, maturation, and gonadal relationships with nutrients. However, nutrients can be required in up to 10 times more under conditions of diseases, stress, and social interactions than under normal culture conditions (TOGUYENI *et al*., 1997). SIGNOR *et al*. (2013) used retinyl acetate Microvit™ A Supra 1000 as the source of vitamin A (0; 3,000; 6,000; 9,000 and 12,000 UI of vitamin A for kg diet) to supplement diets for pacu (66 grams of initial weight) and showed no influence on productive performance in fish cultivated in cages. However, the authors evidenced the need for more research aimed at establishing the requirement of vitamin A in this species.

Because the liver is the storage site of vitamin A, it is considered the main organ related to the metabolism and homeostasis of this vitamin. Its absorption begins in the intestine followed by assimilation in enterocytes linked to crude protein. Retinyl esters are incorporated in the chylomicron and secreted into the bloodstream. Chylomicrons are incorporated through endocytosis into hepatocytes in the liver where retinyl esters are stored, subsequently hydrolyzed, and released as free retinyl; the retinyl carrier protein transfers it to hepatic stellate cells where it is re-esterified into retinyl esters and stored in cytoplasmic lipid droplets (FERNANDEZ and GISBERT, 2011).

The ingestion of vitamin A is important in fish because it acts on differentiation and cellular proliferation, vision, reproduction, embryonic development, immune response (HALVER, 2002), skeletal deformation (LALL and LEWIS-MCCREA, 2007; FERNANDEZ *et al*., 2009), and growth (WESTON *et al*., 2003). Its deficiency can cause skin bleeding, vision problems, changes in the hepatosomatic ratio, and fish mortality (SALEH *et al*., 1995; HAYASHIDA *et al*., 2004; MOREN *et al*., 2004). However, the excess of vitamin A in the diet reduces the productive performance and survival, causes the liver to become yellowish, and reduces hemoglobin and hematocrit levels (HILTON, 1983).

Several studies demonstrate the effects of vitamin A on various metabolic aspects in fish, either in reproduction and larval stages or in growth. However, SIGNOR *et al*. (2013) is the only study reporting that vitamin A does not influence growth performance, chemical composition, blood parameters, and total lipid content in the liver of juvenile pacus (*P. mesopotamicus*) grown in cages and weighing more than 66 grams. Therefore, the authors indicated that further research was needed to evaluate the influence of vitamin A on growth performance in pacu. On the other hand, BUENO *et al*. (2008) reported that the environment used by the researchers in that study (SIGNOR *et al*., 2013) was oligotrophic, which may have compromised the study results. Knowledge about the effects of vitamin A on fish growth is essential for de designing of diet formulations that meet the nutritional requirements of fish (NRC, 2011). An excess, as well as a lack of vitamin A, may impair growth and survival and cause skeletal formation in fish (ORNSRUD *et al*., 2002; HU *et al*., 2006; PEIL *et al*., 2007; FERNANDEZ *et al*., 2008; FERNANDEZ *et al*., 2009).

The aim of the present study was to evaluate the supplementation of vitamin A in diets for pacu fingerlings (*Piaractus mesopotamicus*) in regards to productive performance responses, carcass chemical composition, and hematological and liver histological parameters.

METHODS

The experiment was performed for 102 days in the Aquaculture Laboratory at the State University of Western Paraná in Toledo-PR. This methodology was approved by the Committee on Ethical Conduct for the Use of Animals in Experimentation from the State University of Maringá under protocol number 066/2009.

Preparation and analysis of diets

Prepared mineral and vitamin supplements containing 0, 3,000, 6,000, 9,000, and 12,000 IU of vitamin A per kilogram of diet, through the use of retinyl acetate (Microvit™ A Supra 1000) in the concentration of 1,000,000 IU of vitamin A g^{-1} were used in the experiments. The vitamin was the last ingredient added to the diets (Table 1). The ration was formulated according to the nutritional requirements of 28% crude protein (PB) and 3,000 kcal of digestible energy (ED) kg^{-1} of ration.

The ingredients (corn, soybean, and wheat bran, and poultry viscera and fish meal) were weighed, blended, and ground in a hammer mill until reaching 0.8 mm in diameter. Micronutrients, mineral and vitamin supplements, antifungal, and oil were subsequently added and mixed. The diets were subjected to extrusion producing pellets with 2 mm that were dried in a forced convection oven at

Table 1. Percentage and chemical composition estimated in the basal ration for pacu fingerlings, *Piaractus mesopotamicus*.

Ingredients	%
Corn grain	41.36
Soybean meal	26.77
Poultry viscera meal	14.65
Fish meal	11.44
Wheat bran	3.00
Soybean oil	1.65
Mineral and vitamin supplement(1)	0.50
Sodium chloride	0.30
DL-methionine	0.21
Antifungal (calcium propionate)	0.10
Antioxidant (BHT)	0.02
Total (g)	100
Chemical composition(2)	%
Dry matter	91.00
Protein	28.10
Fat	7.00
Mineral matter	6.40

(1) Assurance levels per kilogram of product: Vit. D_3, 400,000 IU; Vit. E, 30,000 IU; Vit. K3, 2,000 mg; Vit. B1, 4,000 mg; Vit. B2, 4,000 mg; Vit. B6, 2,000 mg; Vit. B12, 8 mg; Folic acid, 1,000 mg; Ca Pantothenate 1,000 mg; Vit. C, 60,000 mg; Biotin, 200 mg; Inositol, 20,000; Niacin, 20,000; Choline, 100,000 mg; Co, 140 mg; Cu, 2,000 mg; Fe, 16,000 mg; I, 200 mg; Mn, 10,000 mg; Se, 80 mg; and Zn, 16,000 mg. (2) Analysis performed in the Laboratory of Food Quality/Unioeste.

55 °C, packaged, labeled, and stored in a refrigerator until use. The fish were fed daily to satiety at 8 and 11 am, and 2 and 5 pm.

Experimental design

A total of 240 fingerlings were used with an average weight of 17.55 ± 3.22 g and 9.29 ± 0.69 cm in total length. They were randomly distributed in five treatments with four replicates; each tank contained 12 fish was considered one experimental unity. Twenty circular 500 L-fiberglass tanks with a conical bottom and constant oxygenation produced by a central air blower were used; these tanks contained a water recirculation system with central and low water outlets. The water circulated through a mechanical and biological filter, and a heating system. Feeding was done *ad libutum* at 8 an 11 am, 2 and 5 pm.

Water quality

The water electrical conductivity (μS cm^{-1}), dissolved oxygen (mg L^{-1}), and pH were monitored weekly while temperature (ºC) was monitored twice daily, in the morning (8 am) and afternoon (5 pm). Water samples were collected in dark polyethylene bottles and evaluated in the Laboratory of Food Quality Control at the State University of Western Paraná, in Toledo-PR for turbidity, ammonia, orthophosphate, and available phosphate. Turbidity was measured using HANNA instruments (Hanna Instruments®); these samples were subsequently filtered using a vacuum pump for the analyses of ammonia, orthophosphate, and dissolved phosphate. The ammonia content was analyzed according to the methodology described by STRICKLAND and PARSONS (1972), and orthophosphate and dissolved phosphate were analyzed according to the methodology described by MACKERETH *et al*. (1978). The average values of water quality parameters were 27.14 ± 0.54 °C; 6.89 ± 0.39 pH; 6.84 ± 0.97 mg L^{-1} dissolved oxygen; 91.0 ± 4.92 μS cm^{-1} electrical conductivity; 0.77 ± 0.13 turbidity; 0.04 ± 0.01 mg L^{-1} ammonia; 0.83 ± 0.01 mg L^{-1} orthophosphate; and 0.80 ± 0.01 mg L^{-1} dissolved phosphate.

Productive performance

The fish were submitted to fasting for 12 h at the end of the experimental period and euthanized with benzocaine at the concentration of 250 mg L^{-1} of water (GOMES *et al*., 2001) for the analyses of productive performance, yield, carcass chemical composition, and liver histology.

All fish were weighed and counted for the calculation of final weight, weight gain (*final weight-initial weight*), daily weight gain (*weight gain/days of cultivation*), final length, condition factor ((*final weight/final length*^{3})**100*), apparent feed conversion (*ration intake/weight gain*), and survival ((*final number of animals/initial number of animals)*100*).

After being weighted, the animals were identified, immersed in ice, and sent to the Laboratory of Fish Technology at the State University of Western Paraná, in Toledo-PR. These fish were subsequently eviscerated for the removal of visceral fat and liver for the calculation of body yield (*weight without viscera*100)/total weight*), the hepatosomatic ratio (*liver weight*100)/total weight*), and visceral fat (*visceral fat weight*100)/total weight*).

Carcass chemical composition

Six eviscerated fish per replicate were frozen for later analysis on carcass chemical composition. Whole fish were stored at -18 °C for the analyses of dry matter, chemical composition, crude protein, lipid, and mineral matter. Dry weight was calculated after dehydration in a forced convection oven at 55 °C. The crude protein analysis was performed by the Kjeldahl method. Lipids were assessed using the Soxhlet method, and the mineral matter was obtained using a muffle furnace at 550 ºC.

Hematology parameters

Eight specimens were collected in each experimental unit for blood evaluation; the fish were anesthetized with benzocaine (75 mg L^{-1} of water) (GOMES *et al*., 2001) and analyzed in the Hematology Laboratory of the State University of Western Paraná, in Toledo-PR. One milliliter of blood was collected from each fish through caudal puncture using a disposable heparinized syringe. These blood samples were used for erythrocyte counts and hemoglobin and hematocrit analysis according to the methodology described by COLLIER (1944) and GOLDENFARB *et al*. (1971), respectively. The hematimetric indexes were calculated based on the average of corpuscular hemoglobin *(hemoglobin*10/erythrocytes*), average of corpuscular hemoglobin concentration (*hemoglobin*100/hematocrit*), and corpuscular volume (*hematocrit*10/erythrocytes*).

Liver histology

Two livers were collected per replicate (8 per treatment), weighted, and macroscopically evaluated in the Laboratory of Animal Histotechniques from the Department of Animal Morphology at the State University of Maringá for the histological analyses. Liver samples were fixed in Bouin's solution (750 mL picric acid, 250 mL formaldehyde, and 50 mL glacial acetic acid) for 24 h, and transferred and stored in 70° GL alcohol solution. These samples were subsequently dehydrated through an ascending alcohol series, diaphanized in xylene, and embedded in paraffin for the production of 6.0 μm thick semi-serial cross-sectional slices using a rotary microtome (Leica RM 2145). These slices were mounted on slides that were stained with hematoxylin-eosin (HE) for the evaluation and description of liver morphology and tissue integrity under a microscope (400X). Fifty images/slides/fish were captured for the quantification of hepatocytes using a high-resolution digital camera (Pro-series®Cybertecnics Average) coupled to an Olympus Bx 41® microscope and image analysis system (Image-Pro Plus® 4.5.1-Media Cybernetics Inc.), totaling 400 images/treatment. The total area in the microscopic field (90,570.13 mm^2) subtracted from the area occupied by the centrilobular vein (9,630.75 mm^2), corresponding to 80,939.25 mm^2 of the counting useful area, was considered as the standard measurement area.

Statistical analyzes

The data were submitted to the test of homogeneity and normality of *Cramer-Von Mises*. The ANOVA regression analysis, applied at the 5% significance level, was used to determine the requirements of pacu fingerlings according to the NRC (2011) using the statistical program SAS (SAS, 2004).

RESULTS

Productive performance

The data demonstrates that the vitamin A supplementation did not influence ($P > 0.05$) the parameters of survival, final length, condition factor, weight eviscerated fish, and visceral fat (Table 2). However, weight gain and apparent feed conversion in pacus were influenced by the diet supplementation with vitamin A.

The quadratic effect was observed on daily weight gain ($y = -6E^{-07}x^2 + 0.0079x + 78.627$, $R^2 = 0.96$) and apparent feed conversion ($y = 9E^{-09}x^2 - 0.0001x + 1.6086$, $R^2 = 0.84$) through the regression analyses; the derivation of the equation showed optimal levels at 6,583 and 5,555 IU of vitamin A per kilogram of diet, respectively (Figure 1).

Carcass chemical composition

In this study, the levels of vitamin A supplemented in the diets for pacu did not show effects on crude protein, lipids, and carcass mineral matter (Table 3).

Hematology parameters

No differences were observed in hematological parameters such as hematocrit, erythrocytes, hemoglobin, corpuscular volume average, corpuscular hemoglobin average, and corpuscular hemoglobin concentration average between different levels of vitamin A supplementation in the diet ($P > 0.05$) (Table 4).

Liver histology

The vitamin A supplementation in the diet for pacu fingerlings showed no effects on the hepatosomatic index and number of hepatocytes ($P > 0.05$) (Table 5) in this study.

Table 2. Productive performance of pacu fingerlings fed with different levels of vitamin A.

Parameters	Vitamin A (IU kg^{-1})					Effect
	0	3,000	6,000	9,000	12,000	
Initial weight (g)	17.45 ± 1.4	17.86 ± 1.9	17.72 ± 1.8	17.86 ± 1.3	17.67 ± 1.0	NS
Survival (%)	95.83 ± 0.3	97.22 ± 4.2	97.20 ± 4.0	100.00	97.90 ± 4.2	NS
Final length (cm)	16.09 ± 0.2	17.38 ± 0.6	15.57 ± 0.7	17.46 ± 0.5	16.83 ± 0.6	NS
Final condition factor	2.56 ± 0.1	2.24 ± 0.1	2.14 ± 0.1	2.10 ± 0.1	2.15 ± 0.1	NS
Weight eviscerated fish (%)	84.06 ± 1.9	83.89 ± 3.2	85.06 ± 1.8	85.76 ± 0.6	85.76 ± 0.4	NS
Visceral fat (%)	1.27 ± 0.3	1.27 ± 0.1	1.53 ± 0.2	1.46 ± 0.2	1.75 ± 0.3	NS

NS = not significant ($P > 0.05$).

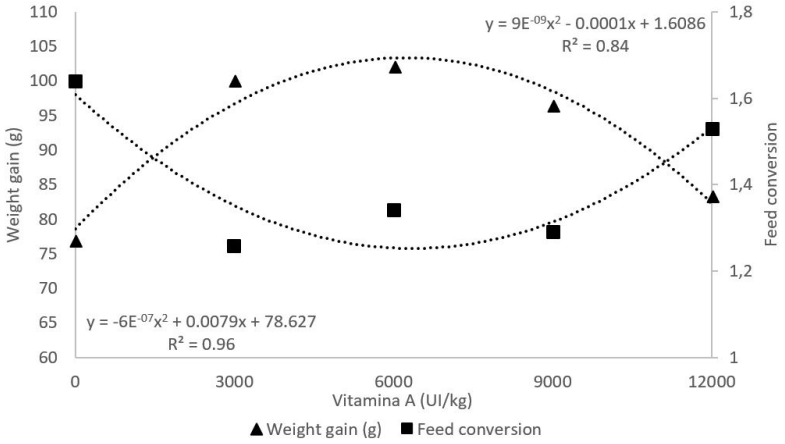

Figure 1. Effect of different levels of vitamin A supplementation in the diet for pacu fingerlings on weight gain and apparent feed conversion.

Microscopically, the hepatic parenchyma observed in all fish regardless of the supplemented levels of vitamin A in the diet showed: i) hepatocytes arranged in continuous strands permeated with sinusoids and converging to the central lobular vein (Figure 2); ii) typical hepatocytes with rounded nuclei in the central position with evident nucleoli, and lightly acidophilic and vacuolized cytoplasm; and iii) larger than normal hepatocytes with periphery displaced nuclei.

Table 3. Carcass chemical composition in pacu fingerlings fed with different levels of vitamin A supplementation in the diet.

Parameters (%)	Vitamin A (IU kg^{-1})					Effect
	0	3,000	6,000	9,000	12,000	
Dry matter	71.48 ± 1.6	71.66 ± 3.3	72.33 ± 0.6	71.14 ± 0.9	72.84 ± 0.6	NS
Crude protein	18.08 ± 0.9	17.30 ± 0.5	17.52 ± 3.1	17.40 ± 1.2	18.78 ± 1.3	NS
Lipid	6.83 ± 1.4	6.49 ± 2.3	5.94 ± 0.9	7.18 ± 0.9	5.81 ± 0.5	NS
Mineral matter	4.43 ± 0.5	4.18 ± 1.6	3.93 ± 0.9	3.80 ± 0.7	3.53 ± 0.7	NS

NS = not significant (P > 0.05).

Table 4. Hematology parameters in pacu fingerlings fed with different levels of vitamin A supplementation in the diet.

Parameters	Vitamin A (IU kg^{-1})					Effect
	0	3,000	6,000	9,000	12,000	
Hematocrit (%)	39.25 ± 1.9	37.95 ± 2.2	38.05 ± 2.2	37.50 ± 1.2	38.75 ± 2.0	NS
Erythrocytes (10^6 µL^{-1})	2.34 ± 0.2	2.37 ± 0.1	2.50 ± 0.4	2.42 ± 0.3	2.28 ± 0.1	NS
Hemoglobin (g dL^{-1})	9.92 ± 0.3	10.04 ± 0.6	9.96 ± 1.2	10.01 ± 0.4	9.75 ± 0.5	NS
VCM (µ^3)	179.09 ± 22.5	161.75 ± 9.7	156.44 ± 27.8	157.14 ± 17.2	172.00 ± 11.7	NS
HCM (pg)	43.35 ± 5.2	42.80 ± 2.6	41.46 ± 11.1	41.90 ± 4.1	43.42 ± 3.2	NS
CHCM (%)	25.33 ± 0.9	26.51 ± 1.7	26.33 ± 4.2	26.74 ± 1.7	25.21 ± 0.7	NS

VCM: Corpuscular volume average; HCM: Corpuscular hemoglobin average; CHCM: Corpuscular hemoglobin concentration average; NS = not significant (P > 0.05).

Table 5. Hepatosomatic ratio and number of hepatocytes in the liver of pacu fingerlings fed with different levels of vitamin A supplementation.

Vitamin A (IU kg^{-1})	Hepatosomatic Index (%)	Hepatocytes (cells area^{-1})*
0	1.29 ± 0.1	257.6±47.7
3,000	1.04 ± 0.3	256.9±25.4
6,000	1.10 ± 0.1	227.4±23.9
9,000	0.98 ± 0.1	249.0±21.3
12,000	1.12 ± 0.1	284.9±36.7
Effect	NS	NS

*area corresponding to 80,939.25 mm^2; NS = not significant (P > 0.05).

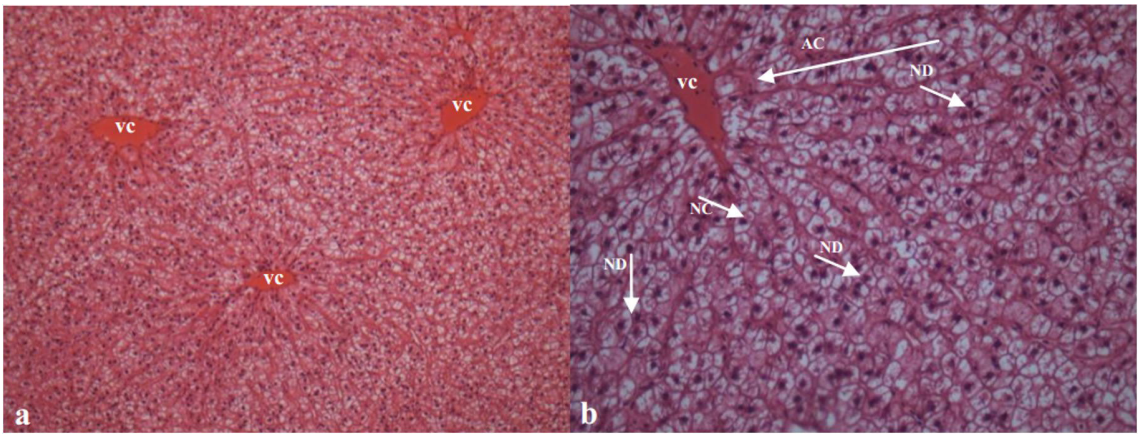

Figure 2. Liver characteristics of pacu juveniles fed diets supplemented with vitamin A. Lobular center vein (VC); cordonal arrangement of hepatocytes (arrow AC); central nucleus of the hepatocyte (arrow NC); nucleus displaced to the periphery of the hepatocyte (arrow ND).

DISCUSSION

We observed a quadratic effect in weight gain and apparent feed conversion as a function of the vitamin A supplementation in the diet, demonstrating that vitamin A influences the metabolism and consequently the productive performance of fish. The fact of SIGNOR *et al*. (2013) evaluated different levels (0; 3,000; 6,000; 9,000, and 12,000 UI vitamin A per kilogram of diet) of vitamin A in pacu cultivated in cages and did not observe differences in weight gain may be related to the short period of cultivation in that study; the authors of that study explained that this short period of cultivation did not allow the observation of negative effects related to a lack or excess of vitamin A in the diet. It can be inferred that in the study by SIGNOR *et al*. (2013), the fish consumed natural microorganisms from the culture environment, which complemented the nutritional requirement because that was an oligotrophic environment (BUENO *et al*., 2008; BARBIERI and BONDIOLI, 2015).

Because the Nile tilapia is an omnivorous species and similar to pacu, the optimal levels of vitamin A observed in pacus could be similar to those established in Nile tilapia, which varies between 3,802 and 6,970 IU of vitamin A per kilogram of diet (SALEH *et al*., 1995; HU *et al*., 2006; CAMPECHE *et al*., 2009). PEIL *et al*. (2007) determined the requirement of 3,955 UI per kilogram of diet when evaluating vitamin A supplementation in diets for silver catfish larvae.

Studies that evaluated vitamin A dose responses report that mortality is common when there is a vitamin A deficiency in the diets (ORNSRUD *et al*., 2002; HU *et al*., 2006; PEIL *et al*., 2007; CAMPECHE *et al*., 2009). In this study, the presence or absence of vitamin A supplementation in the diet did not interfere with the survival of pacus, which corroborates the data observed by SIGNOR *et al*. (2013) in the same species. Likewise, GUO *et al*. (2010) did not observe an effect of vitamin A supplementation in the diet on the survival of tilapia fingerlings; these authors observed 98.57% survival in fish that received a diet without vitamin A supplementation. Conversely, the survival rate of 48% in fish receiving the control diet and 73.68% in fish receiving diets with 15,000 IU of vitamin A per kilogram of diet was observed in silver catfish fingerlings (*Rhamdia quelen*) (PEIL *et al*., 2007).

The deposition of lipids in the viscera is characteristic of species when the excess of a nutrient is stored as fat. This storage can reach 7.97% in the abdominal cavity and 3.30% in the carcass of animals with 850 g in weight (SIGNOR *et al*., 2010), and 7.02% and 9.0% in fish grown in net tanks and approximate weight of 1,250 g (BITTENCOURT *et al*., 2010). Dietary vitamin A supplementation did not influence the deposition of visceral fat for pacu (SIGNOR *et al*., 2013) and reduced for tilapia hybrids (*Oreochromis niloticus* x *O. aureus*) (HU *et al*., 2006) and salmon (ORNSRUD *et al*., 2002).

The vitamin A supplementation in the diet did not alter parameters such as dry matter, crude protein, lipids, and mineral matter in carcasses of pacu. SIGNOR *et al*. (2013) did not observe the effect of vitamin A on the chemical composition of carcasses of pacu. However, the effects of vitamin A supplementation on the chemical composition of the carcass may present divergent effects due to metabolic characteristics related to the physiology and different feeding habits between different species, which makes it difficult to compare results.

The erythrogram results were within the standards established for pacus as described by RANZANI-PAIVA *et al*. (1999), TAVARES-DIAS *et al*. (1999), and TAVARES-DIAS *et al*. (2002). However, in this study, no differences were observed between erythrogram values from pacu fingerlings fed with different levels of vitamin A supplementation in the diet. Similar results have been reported in hematocrit (HERNANDEZ *et al*., 2007; GUO *et al*., 2010) and hemoglobin (GUO *et al*., 2010). Conversely, GOSWAMI and DUTTA (1991) observed reduced values of hemoglobin, erythrocytes, and hematocrit with increasing vitamin A levels in the diet.

The hepatosomatic indexes observed in pacus were similar to those reported by SIGNOR *et al*. (2013). TAVARES-DIAS *et al*. (2000) reported that the hepatosomatic index of this species might range from 0.98 to 1.23 depending on body weight, gender, and maturation status. In this study, all fish presented similar liver characteristics with hepatic tissue irregularities regardless of the levels of vitamin A supplementation. Similar results were reported by SOUZA *et al*. (2001) and FUJIMOTO *et al*. (2008) in studies with pacu (*P. mesopotaminus*). Most studies about vitamin A in fish report that the supplementation does not influence the hepatosomatic index in fish (HEMRE *et al*., 2004; LIÑÁN-CABELLO and PANIAGUA-MICHEL, 2004; HERNANDEZ *et al*., 2007; FONTAGNÉ-DICHARRY *et al*., 2010); Yet even though this organ is a local storage of vitamin A in fish (CAMPECHE *et al*., 2009; FERNANDEZ and GISBERT, 2011) the studied supplementation levels did not alter the liver size.

The variation in the number of hepatocytes present in the hepatic parenchyma reflects the effect of the diet on the body metabolism, although no variation was observed in pacus. Vitamin A is a liposoluble product that, when absorbed in the liver, can be stored in the tissue (NRC, 2011) prolonging the appearance of clinical signs of deficiency. A prolonged experimental period is fundamental for the appearance of such clinical signs (MOREN *et al*., 2004). Therefore, variations in the liver of fish are relevant to provide information that can contribute to the development of diets that meet the nutritional requirements of each cultivation phase (CABALLERO *et al*., 1999; BOLLA *et al*., 2011).

CONCLUSION

We concluded that the supplementation of at least 5,555 IU of vitamin A per kilogram of diet is needed to improve the performance of pacu fingerlings (*P. mesopotamicus)*.

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