

Anais da Academia Brasileira de Ciências (2013) 85(1): 385-393 (Annals of the Brazilian Academy of Sciences) Printed version ISSN 0001-3765 / Online version ISSN 1678-2690 www.scielo.br/aabc

Growth and hematology of juvenile pacu *Piaractus mesopotamicus* (Holmberg 1887) fed with increasing levels of vitamin E (DL-α-tocopheryl acetate)

RICARDO Y. SADO¹, ÁLVARO J.A. BICUDO² and JOSÉ E.P. CYRINO³

 ¹Universidade Tecnológica Federal do Paraná, Campus de Dois Vizinhos, Estrada para Boa Esperança, Km 04, 85660-000 Dois Vizinhos, PR, Brasil
 ²Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Garanhuns, Avenida Bom Pastor, s/n, 55296-901 Garanhuns, PE, Brasil
 ³Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Caixa Postal 9, 13418-900 Piracicaba, SP, Brasil

ABSTRACT

Intensive fish production systems are characterized by 100% artificial feeding, so any dietary imbalances or deficiencies may lead to diseases outbreaks and economic losses. This study was set out to determine the effects of increasing levels of dietary vitamin E on growth and hematology of juvenile pacu. Fishes were fed for 90 days, twice a day until apparent satiation with semi-purified diets containing 0.0; 25; 50; 150; 300 or 600 mg.kg⁻¹ diet DL- α -tocopheryl acetate in a completely randomized design trial (n=4); biometrical and hematological data were collected and analyzed. Fishes fed with vit E diet (150 mg.kg⁻¹) showed higher (p<0.05) weight gain and specific growth. Hematocrit, erythroblast number and total plasma protein were increased (p<0.05) in fishes fed diet with no vit E diet. Vitamin E supplementation in artificial diets for pacu is essential for growth and maintenance of normal erythropoiesis.

Key words: fish nutrition, hematology, Piaractus mesopotamicus, vitamin E.

INTRODUCTION

Global fish consumption has doubled since the 1970s and it is still growing (Naylor and Burke 2005). Following this trend, Latin America has shown increased aquaculture production and *per capita* consumption (FAO 2009), as well as intensification of fish production systems, characterized by high biomass and use of balanced, pelletized or extruded diets to meet fish nutritional requirements. Therefore, imbalanced artificial diets would lead to significant economical losses due to nutritional deficiencies or diseases outbreaks.

Corresponding to: Ricardo Yuji Sado email: ricardoysado@utfpr.edu.br Provided by exogenous source (i.e., diet), vitamins are essential for the metabolism in fishes playing an important role on biochemical reactions related to growth and health (Halver 2002, NRC 2011, Tocher et al. 2003). Within the fat-soluble vitamins (A, D, E, and K), vitamin E is one of the most studied in fish dietetics.

Vitamin E has antioxidant properties, protecting cellular macromolecules (DNA, proteins and fat acids) against free radical oxidative processes during normal cellular metabolism or humoral and cellular defense mechanisms. In addition, this nutrient can present immunostimulatory function such as eliciting increased leukocyte production and phagocytic activity (Chen et al. 2004, Pulsford et al. 1995, Wise et al. 1993). Effects of vitamin E have been determined for several, economically important fish species such as gilthead seabream *Sparus aurata* (Montero et al. 1999), grouper *Epinephelus malabaricus* (Lin and Shiau 2005), rainbow trout *Oncorhynchus mykiss* (Kiron et al. 2004, Pearce et al. 2003, Trenzato et al. 2007), Atlantic salmon *Salmo salar* (Hamre et al. 1997, 2004), red drum *Sciaenops ocellatus* (Peng and Gatlin 2009) and channel catfish *Ictalurus punctatus* (Wise et al. 1993). Analysis of blood components may provide important information regarding 64 general condition and possible effects of vitamin E on fish.

Pacu (*Piaractus mesopotamicus*) is a neotropical freshwater Characin native of Parana, Paraguay and Uruguay basins. Because of its herbivorous/ omnivorous habits, high growth rates, good meat quality, consumer acceptance and suitability for sports fishery, the specie is widely used in aquaculture (Jomori et al. 2005, Urbinati and Gonçalves 2005). Few studies regarding the effect of dietary vitamin E for pacu are available (Belo et al. 2005a, b, Garcia et al. 2007). This study was set out to evaluate the effects of increasing levels of dietary vitamin E on growth and hematology of pacu juveniles.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND ANIMALS

Trials were set up in a closed water recirculation system, with supplemental aeration and emergency oxygenation systems. Water quality parameters such as pH (7.60 \pm 0.20), dissolved oxygen (5.8 \pm 0.30 mg.L-1), ammonia (\leq 0.5 mg.L⁻¹) and temperature (30.3 \pm 1.8 °C) remained within acceptable values for the specie (Urbinati and Gonçalves 2005). A 12h light/12h dark photoperiod was maintained. Juvenile pacu (7.83 \pm 0.04 g) obtained from commercial hatchery were acclimatized to the experimental conditions for seven days feeding on a 40% crude protein (CP) commercial diet.

EXPERIMENTAL DIETS

The basal, experimental semi-purified diets were made according 88 to the species' requirements (Table I). The vitamin and mineral mix did not contain vitamin E; dietary vitamin levels were set according to vitamin E activity of the dietary source (ROVIMX E 50 Adsorbate Roche[®]: 50% vitamin E activity). Ingredients were weighed, homogenized and mixed, moistened with distilled water (25-30%) and pelleted (2.0 mm) in a mincer. Prepared rations were dried in a forced ventilation oven at 45 °C for 24h; the dried pellets were packed in black plastic bags and stored at -4 °C until use. Diets were analyzed for vitamin E contents at a commercial laboratory (CBO Assessoria & Analises; Campinas, Sao Paulo, Brazil). The concentrations of vitamin E of the experimental diets are presented in Table II.

 TABLE I

 Composition of basal semi-purified diet for *P. mesopotamicus*.

Ingredient	%
Starch	34.24
Albumin	30.86
Cellulose	13.0
Gelatin	7.0
Dextrin	5.0
Bicalcium phosphate	4.0
Soy oil	3.87
Vitamin and mineral mix1 (vit E free)	2.0
BHT (antioxidant)	0.02
Calculated composition	%
Crude protein	30.1
Dry matter	92.66
Ether extract	3.2
Ash	5.66

¹Vitamin and mineral supplement composition (Supre Mais[®]): vitamins A (1.200.000 IU); B1 (4.800 mg); B12 (4.800 mg); B2 (4.800 mg); B6 (4.800 mg); C (48 g); D3 (200.000 IU); 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 (3.000 mg); Manganese (20.000 mg); Zinc (30.000 mg); Vehicle (1.000 g).

 TABLE II

 Expected and detected vitamin E levels in experimental diets.

Treatments	vit E* expected level (mg.kg ⁻¹)	vit E* detected level (mg.kg ⁻¹)
Ι	0.0	Not detected**
II	25	32.79
III	50	50.33
IV	150	136.40
V	300	246.17
VI	600	457.46

* Vitamin E source: DL- α -tocopheryl acetate (50% activity) – ROVIMIX E 50 adsorbate - Roche[®]

** Under quantification limit: 2.0 mg.kg-1

ROUTINE PROCEDURES

After acclimation period, fishes randomly assigned to 60 L cages (20 fish per cage) were fed a vitamin E-free diet for 15 days to zero vitamin E reserves and as well as diets containing 0.0; 25; 50; 150; 300 and 600 mg.kg⁻¹ vit E until apparent satiation, twice a day (07:00 and 16:00 h) for 90 days, in a completely randomized experimental design (n=4). At the end of experimental period, fish were fasted for 24h, anesthetized with alcoholic solution of benzocaine at 50 mg.L⁻¹, weighted, measured, and had blood samples drawn for analysis. Growth parameters were evaluated according to Tacon 107 (1990) as follows:

- Weight gain (WG) WG = FW - IW
- Feed conversion ratio (FCR) $FCR = \frac{\text{feed consumption}}{\text{weight gain}}$
- Daily feed consumption (FC) FCR = $\frac{\text{feed consumption}}{t}$
- Specific growth rate (SGR) SGR = $100 \times \frac{(\ln FW \ln IW)}{t}$

where: FW = final weight (g); IW= initial weight (g); t = experimental time (days).

Blood samples were drawn from the caudal vein using sterilized syringes and 10% EDTA-coated needles. Red blood cells (RBC) count was performed in Neubauer chamber using the Natt and Herrick (1952) diluent; hematocrit evaluation followed the microhematocrit method of Goldenfarb et al. (1971); hemoglobin concentration was performed following the cyanometahemoglobin method (Blaxhall and Daisley 1973). Hematimetric indexes calculated were mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Wintrobe 1934). Total plasma protein concentrations were determined using a portable refractometer (WZ-301/Protein 0.0-12 g.dL⁻¹) after blood centrifugation and plasma collection (Sado et al. 2008). Plasma glucose was determined by the enzymatic method using a standard kit (GLICOSE GOD-PAP Liquid Stable Mono Reagente, LABORLAB[®]; Guarulhos, Sao Paulo, Brazil).

Blood smears from individual fish were stained with May-Grünwald-Giemsa (Rosenfeld 1947) and examined under light microscopy using an oil immersion objective for differential leukocyte count, and white blood cell (WBC), thrombocyte and erythroblast counts. White blood cell (WBC), thrombocyte and erythroblast count were performed by indirect method (Garcia et al. 2007, Sado et al. 2010) as follow: WBC $(\mu L^{-1}) =$ (leukocytes number in blood smear x erythrocyte number. μL^{-1}) \div 2,000 erythrocytes counted in the blood smear; Thrombocytes $(\mu L^{-1}) =$ (thrombocytes number in blood smear x erythrocyte number. μ L⁻¹) ÷ 2,000 erythrocytes counted in the blood smear; Erythroblast $(\mu L^{-1}) = (erythroblast$ number in blood smear x erythrocyte number. μ L⁻¹) \div 2,000 erythrocytes counted in the blood smear.

Data were submitted to ANOVA. Significant differences between treatment means were further compared by Tukey test (α =0.05) (Steel and Torrie 1980).

RESULTS AND DISCUSSION

Fishes performance and survival data are summarized in Table III. The acceptability of experimental diets was adequate in all treatments. Weight gain and specific growth rate were affected by dietary vitamin E levels (p<0.05). Fishes fed diet with 136.4 mg.kg⁻¹ of vitamin E diet showed better weight gain when compared to fishes fed with no vitamin E. Specific growth rate showed higher values in fishes fed on diet containing 136.4; 246.17 and 457.46 mg vit E per kg when compared to no vit E treatment. Dietary vitamin E levels influenced (p<0.05) hematological parameters and blood biochemistry (Table IV). Hematocrit values were higher in fishes fed with no vitamin E (32.6%) and 50.33 mg.kg⁻¹ vitamin E diet (32.3%) when compared to values recorded for fishes fed with 457.46 mg.kg⁻¹ vitamin E diet (30.1%). Total plasma protein concentrations were higher (5.5 g.dL⁻¹; p<0.05) for fishes fed with no vitamin E diets than fishes fed with diets of 32.79; 136.4; 246.17 and 457.46 mg vit E per kg.

Significantly higher number of erythroblasts was registered for fish fed with diet devoid of

TABLE III

Means and standard deviation (SD) of individual weight gain (WG), feed consumption (FC), feed conversion rate (FCR), specific growth rate (SGR) and survival rate (SR) of juvenile pacu *P. mesopotamicus* fed increasing levels of dietary vitamin E.

Vit. E*			Growth parameters		
(mg.kg ⁻¹ diet)	WG (g)	FC (g)	FCR	SGR (%)	Survival (%)
0.0	64.17±6.0 ^a	75.02±7.0	1.17±0.08	3.25±0.12 ^a	93.3±6.6
32.79	73.96±6.7 ^{ab}	83.55±4.6	1.13±0.07	3.44±0.11 ^{ab}	90.0±3.8
50.33	73.63±8.0 ^{ab}	82.84±7.5	1.08±0.05	3.49±0.13 ^{ab}	91.6±6.3
136.4	86.50±12.5 ^b	95.34±14.1	1.10±0.10	3.65 ± 0.18^{b}	84.4±16.7
246.17	85.36±12.2 ^{ab}	91.47±11.9	$1.07{\pm}0.08$	3.63 ± 0.19^{b}	91.1±3.8
457.46	84.77±0.9 ^{ab}	91.84±2.2	1.08±0.03	3.63 ± 0.00^{b}	88.8±13.8

Different letters superscript at same columns denote differences by Tukey test (α =0.05).

* Vitamin E source: DL-α-tocopheryl acetate (50% activity) - ROVIMIX E 50

adsorbate - Roche®

TABLE IV

Hematological parameters (µ ± SD) of juvenile pacu *P. mesopotamicus* supplemented with increasing levels of dietary vitamin E.

Variables -	Vitamin E* (mg.kg ⁻¹ diet)					
	0.0	32.79	50.33	136.4	246.17	457.46
RBC 10 ⁶ .µL ⁻¹	1.46±0.2	1.43±0.3	1.36±0.2	1.35±0.2	1.31±0.2	1.31±0.2
Htc %	32.6±1.1 ^a	31.5 ± 1.7^{ab}	32.3±1.9 ^a	31.5 ± 1.8^{ab}	$314{\pm}17^{ab}$	$30.1{\pm}1.2^{b}$
Hb g.dL ⁻¹	10.4±0.7	10.2±0.6	10.3±0.7	10.2±0.9	9.7±0.7	10.0±0.8
MCV <i>f</i> L	229.5±42.5	226.3±41.0	239.6±27.0	239.5±32.3	245.5±43.7	237.7±41.5
MHC pg.cell ⁻¹	72.7±12.0	73.8±14.5	76.3±9.8	76.9±8.9	77.8±14.4	78.5±11.8
MCHC g.dL ⁻¹	31.8±1.5	32.6±2.3	31.8±1.9	32.2±1.6	31.8±3.1	33.2±2.0
Prtn g.dL ⁻¹	5.5±0.4 ^a	5.0±0.3 ^{bc}	5.3±0.3 ^{ab}	5.0±0.4 ^{bc}	4.9±0.4 ^{bc}	4.6±0.4 ^c
Gluc mg.dL ⁻¹	71.9±11.5	76.8±8.8	79.6±14.6	67.8±10.1	84.0±22.8	84.9±21.1

Different letters superscript at same lines denote differences by Tukey test (α =0.05).

* Vitamin E source: DL-α-tocopheryl acetate (50% activity) – ROVIMIX E 50

adsorbate - Roche[®], RBC: Red Blood Cell, Htc: hematocrit, Hb: hemoglobin concentration, MCV: mean corpuscular volume, MHC: mean hemoglobin concentration, MCHC: mean corpuscular hemoglobin concentration, Ptrn: total plasma protein concentration Gluc: plasma glucose concentration

vitamin E in comparison to fish fed with diets containing increasing levels of vitamin E (Fig. 1). No effect of dietary vitamin E supplementation 159 was found on WBC and thrombocyte count, as well as on differential leukocyte count (Table V). Diet and nutrition can influence growth and disease resistance of domestic and farm animals (Alcorn et al. 2003, Blazer 1991, Landolt 1989, Sitja-Bobadilla and Pérez-Sanchez 1999). In this study, fishes fed with a vitamin E-free diet had poor growth

 TABLE V

 Hematological parameters (µ ± SD) of juvenile pacu P. mesopotamicus supplemented with increasing levels of dietary vitamin E.

Variables	Vitamin E* (mg.kg ⁻¹ diet)					
	0.0	32.79	50.33	136.4	246.17	457.46
Thro.µL ⁻¹	29,642±7,612	24696±6856	24,494±4,249	26,144±4935	22,165±5,474	24,747±6,646
WBC.µL ⁻¹	6,208±2,529	6,111±3,078	5,283±1,919	7,620±2,399	6,774±2,301	$5,355\pm 2,008$
Lym.µL ⁻¹	4,373±1,876	4,156±2,429	3,502±1,603	5,597±1,722	5,091±2,146	3,786±1,652
Mon.µL ⁻¹	244±121	424±249	390±215	412±222	340±211	362±163
Neu.µL ⁻¹	924±309	922±416	1,013±412	922±405	1,011±518	952±378
Eos.µL ⁻¹	194±176	147±144	259±178	233±140	191±100	141±117
SGC.µL ⁻¹	71±68	80±76	59±76	82±54	57±56	113±82

* Vitamin E source: DL-α-tocopheryl acetate (50% activity) - ROVIMIX E 50

adsorbate - Roche[®], Thro: thrombocytes, WBC: White blood cell, Lym: lymphocytes, Mon: monocytes, Neu: neutrophils, Eos: eosinophils, SGC: special granulocytic cells

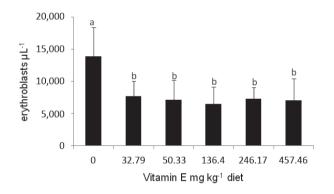


Fig. 1 – Erythroblast number ($\mu \pm SD$) of juvenile pacu *P*. *mesopotamicus* fed increasing levels of dietary vitamin E. Different letters above columns indicate differences by Tukey test (α =0.05).

and weight gain in comparison to fishes fed with diets supplemented with vitamin E. The effect of dietary vitamin E on fish growth still steers controversy. Significant positive effects of increased dietary contents of vitamin E on fish growth have been described by several authors for different species, such as Atlantic salmon (Hamre et al. 1997), rainbow-trout (Pearce et al. 2003, Trenzato et al. 2007), Chinook salmon, *Oncorhynchus tshawytscha* (Thorarinsson et al. 1994), grouper (Lin and Shiau 2005) and rohu (Sau et al. 2004), and poor growth performance was also registered for fishes fed with vitamin E-deficient diets, that is, vitamin E plays an important role in fish dietetics. Like any other animal, fish cannot synthesize vitamin E (Peng and Gatlin 2009).

However, many authors reported no effects of vitamin E supplementation on growth in some fish species, such as the golden shiner, Notemigonus crvsoleucas (Chen et al. 2004), channel catfish (Gaylord et al. 1998, Wise et al. 1993), gilthead seabream (Montero et al. 1999), Nile tilapia (Lim et al. 2010), rainbow-trout (Clerton et al. 2001, Kiron et al. 2004), Atlantic salmon (Hardie et al. 1990, Poston et al. 1976) and hybrid striped bass (Trushenski and Kohler 2008). Belo et al. (2005b) also did not report effects of vitamin E supplementation on growth performance of pacu, possibly as a consequence of fish size - initial average weight 7.8 g in the current study against 96.4 g in Belo et al. (2005b) experiment. As a matter of fact, vitamin E is a fat-soluble nutrient that can

be stored in liver tissue (Halver 2002). Therefore; larger fish may stand longer periods feeding on diets with low or zero vitamin E contents, relying and using vitamin E deposited in body lipids for growth and maintenance of body functions.

In exception of poor growth in fishes fed with the vitamin E-deficient diet, no clinical signs of vitamin deficiency were recorded. Vitamin E deficiency signs in fishes are characterized by darkened skin (Chen et al. 2004) or lack of pigmentation (Hamre et al. 1997), dystrophy and necrosis of epaxial muscles (Chen et al. 2004), and hematological disorders (Chen et al. 2004, Hamre et al. 1997, Pearce et al. 2003, Poston et al. 1976, Wise et al. 1993). Although vitamin E concentrations on fish tissues were not determined, the authors presume that a 15-day withdrawal period in addition to experimental time was not sufficient to decrease vitamin E reserves of pacu; since the same was reported by Hardie et al. (1990) for Atlantic salmon. Belo et al. (2005b) and Garcia et al. (2007) also did not report clinical signs of vitamin deficiency in pacus running 90day feeding trials, and Clerton et al. (2001) also did not report clinical signs on rainbow trout feeding on vitamin E-free diets for 80 days.

Similar to data herein reported no effects of dietary vitamin E on number and proportion of leukocytes was reported by Garcia et al. (2007) for pacu, Lim et al. (2010) for Nile tilapia, and Hardie et al. (1990) for Atlantic salmon. However, increasing and decreasing number of defense cells was reported for fishes fed with vitamin E-deficient diets by Chen et al. (2004) for golden shiner and Lin and Shiau (2005) for the grouper. Although Chen et al. (2004) reported increasing number of leukocytes and thrombocytes, when differential leukocyte count was considered, a decreasing number of lymphocyte was observed. Decreasing lymphocyte number could be a consequence of membrane fragility and cell lysis and/or lymphocyte migration to degenerated muscular tissue, ordinarily observed in fishes showing vitamin E deficiency signs.

No evaluation of the immunological status of fishes' immunological status was herein performed. However, significant effects of vitamin E on their immune systems of fish have been already soundly demonstrated (Chen et al. 2004, Hardie et al. 1990, Kiron et al. 2004, Lin and Shiau 2005, Lygren et al. 2008, Montero et al. 1999, Pearce et al. 2003, Wise et al. 1993). Even thought there were no significant differences in phagocyte numbers, possibly as a result of the high variation, values of monocytes count increased in absolute values in fishes fed with diet of increasing vitamin E contents, regarded a sign of immunity stimulation, given that in in vivo fish inflammatory response studies, macrophages derived from blood circulating monocytes have been reported to differentiate into multinucleated giant cells (Sado and Matushima 2008). The macrophage recruitment and giant cell formation in pacu seems to be strongly related to dietary vitamin E supplementation (Belo et al. 2005b).

Vitamin E protects cell membranes against lipid peroxidation. Dietary vitamin E deficiency in fish increases deformities of membranes and fragility of erythrocytes, easing hemolysis (Halver 2002), and reducing cell survival time, leading to hematological disturbs such as decreased hematocrit values and hemoglobin concentrations (Chen et al. 2004, Pearce et al. 2003, Poston et al. 1976, Thorarinsson et al. 1995). However, no differences on hemoglobin concentrations and RBC were observed between treatments, despite the higher values for RBC found in literature regarding pacu (Martins et al. 1995, Ranzani-Paiva et al. 1998/1999, Tavares-Dias et al. 1999, 2002, Tavares-Dias and Matagueiro 2004). Elevated hematocrit values were herein reported for fishes fed with vitamin E-deficient diets; similar results were reported by Garcia et al. (2007).

Fishes fed with vitamin E deficient diet presented higher erythroblasts number. Immature cells are an ordinary feature of fish blood circulation and typically seen in blood smears under light microscopy (Esteban et al. 2000). In some pathological conditions the number of immature cells can be elevated, as observed in this study and also reported by Poston et al. (1976) and Garcia et al. (2007). A compensatory effect can be seen as a decrease on erythrocytes life time and the consequent release of more immature cells to the blood stream. The red cell maturation process involves chromatin condensation, increase on hemoglobin concentrations and decrease on nuclear and cell size (Esteban et al. 1989). Erythroblasts, i.e. young erythrocytes, are larger than mature red cells. Therefore, an increase in numbers of those cells in fish blood circulation would explain the high hematocrit values found in the current experiment and by Garcia et al. (2007) in fishes fed with vitamin E-deficient diets.

Physiological status can be determined through hematological and biochemical parameters. Experimental procedures and rearing conditions can be deemed adequate since no differences on plasma glucose concentrations were found. A close relationship can be found between total plasma protein concentrations and both protein metabolism and nutritional status (Coles 1984). Elevated values found in fishes fed on diets with no vitamin E supplementation are similar to results from Poston et al. (1976), suggesting cellular protein release through erythrocyte hemolysis.

Conflicting results demonstrate that, in fish, ideal dietary vitamin E concentration for growth and health maintenance depend on several factors, such as vitamin type and source (Norouzitallab et al. 2009, Trushenski and Kohler 2008), production system (Gaylord et al. 1998), and nutrient interactions (Chaiyapechara et al. 2003, Chen et al. 2004, Garcia et al. 2007, Hamre et al. 1997, Jaramillo et al. 2009, Kiron et al. 2004, Lin and Shiau 2005, Montero et al. 1999, Thorarinsson 254 et al. 1994). 255 Vitamin E is a key nutrient for growth and erythropoiesis of pacu. The compensatory effect demands more energy and

protein to the formation of new blood cells which could impair fish growth. Studies about vitamin E supplementation effects in pacu are scarce, do not reflect its economic importance on neotropical aquaculture and shall be fostered.

ACKNOWLEDGMENTS

Authors are indebted to FINEP for financial support (FINEP-FUSP 01.06.0407.00) and FAPESP for doctoral scholarships granted to RY Sado (Proc. 05/51967-2) and AJA Bicudo (Proc. 05/51968-9). JEP Cyrino is a CNPq research scholar.

RESUMO

Sistemas intensivos de produção utilizam 100% de dietas artificiais sendo que, qualquer imbalanço ou deficiência de algum nutriente pode ocasionar surtos de doenças e perdas econômicas. O presente estudo determinou o efeito de níveis crescentes de vitamina E na dieta sobre o desempenho e hematologia de juvenis de pacu. Os peixes foram alimentados por 90 dias até aparente saciedade com dietas semi purificadas contendo 0.0; 25; 50; 150; 300 ou 600 mg.kg⁻¹ de DL-α-tocoferil-acetato, rações em um delineamento experimental inteiramente casualizado (n=4). Parâmetros de desempenho e hematológicos foram coletados e analisados. Peixes alimentados com ração de 150 mg.kg⁻¹ de vit E apresentaram maior ganho de peso (p<0,05) e taxa de crescimento específico. Hematócrito, número de eritroblastos e proteina total plasmática foram maiores (p<0.05) nos peixes alimentados com a dieta insenta de vitamina E. O suplemento de vitamina E em dietas artificiais é essencial para o crescimento e a manutenção da eritropoiese nos valores normais para a espécie.

Palavras-chave: nutrição de peixes, hematologia, *Piaractus mesopotamicus*, vitamina E.

REFERENCES

ALCORN SW, PASCHO RJ, MURRAY AL AND SHEARER KD. 2003. Effects of ration level on immune function in Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 217: 529-545.

- BELO MAA, FENERICK Jr J, SOARES VE AND MORAES FR. 2005a. Suplementação com DL-α acetate de tocoferila e parasitismo por *Anacanthorus penilabiatus* (Monogea: Dactylogyridae) em *Piaractus mesopotamicus* (Osteichthyes: Characidae). Acta Sci Anim Sci 27(1): 73-79.
- BELO MAA, SCHALCH SHC, MORAES FR, SOARES VE, OTOBONI AMMB AND MORAES JER. 2005b. Effect of Dietary Supplementation with Vitamin E and Stocking Density on Macrophage Recruitment and Giant Cell Formation in the Teleost Fish, *Piaractus mesopotamicus*. J Comp Pathol 133: 146-154.
- BLAXHALL PC AND DAISLEY KW. 1973. Routine hematological methods for use with fish blood. J Fish Biol 5: 771-781.
- BLAZER VS. 1991. Piscine Macrophage Function and Nutritional Influences: A Review. J Aquat Anim Health 3(2): 77-86.
- CHAIYAPECHARA S, CASTEN MT, HARDY RW AND DONG FM. 2003. Fish performance, fillet characteristics, and health assessment index of rainbow trout (*Oncorhynchus mykiss*) fed diets containing adequate and high concentrations of lipid and vitamin E. Aquaculture 219: 715-738.
- CHEN R, LOCHMANN R, GOODWIN A, PRAVEEN K, DABROWSKI K AND LEE KJ. 2004. Effects of dietary vitamins C and E on alternative complement activity, hematology, tissue composition, vitamin concentrations and response to heat stress in juvenile golden shiner (*Notemigonus crysoleucas*). Aquaculture 242: 553-569.
- CLERTON P, TROUTAUD D, VERLHAC V, GABAUDAN J AND DESCHAUX P. 2001. Dietary vitamin E and rainbow trout (*Oncorhynchus mykiss*) phagocytic functions: effect on gut and on head kidney leucocytes. Fish Shellfish Immun 11: 1-13.
- COLES EH. 1984. Função hepática. In: COLES HE (Ed), Patologia Clínica Veterinária, 3^a ed., Ed. Manole, São Paulo, SP, Brasil, p. 185-219.
- ESTEBAN MA, MESEGUER J, AYALA AG AND AGULLEIRO B. 1989. Erytropoiesis and Thrombopoiesis in the Head-Kidney of Sea Bass (*Dicentrarchus labrax* L.): An Ultrastructural Study. Arch Histol Cytol 52(4): 407- 419.
- ESTEBAN MA, MUÑOZ J AND MESEGUER J. 2000. Blood Cells of Sea Bass (*Dicentrarchus labrax* L.). Flow Cytometric and Microscopic Studies. Anat Rec 258: 80-89.
- FAO. 2009. The State of World Fisheries and Aquaculture. FAO Fisheries Department, Rome, Italy, 196 p.
- GARCIA F, PILARSKI F, ONAKA EM, MORAES FR AND MARTINS ML. 2007. Hematology of *Piaractus mesopotamicus* fed diets supplemented with vitamins C and E, challenged by *Aeromonas hydrophila*. Aquaculture 271: 39-46.
- GAYLORD TG, RAWLES SD AND GATLIN III DM. 1998. Reevaluation of vitamin E supplementation of pratical diets for channel catfish, *Ictalurus punctatus*, production. Aquacult Nutr 4: 109-114.
- GOLDENFARB PB, BOWYER FP, HALL E AND BROSIOUS E. 1971. Reproducibility in the hematology laboratory: The microhematocrit determination. Am J Clin Pathol 56: 35-39.

- HALVER JE. 2002. The Vitamins. In: HALVER JE AND HARDY RW (Eds), Fish Nutrition, 3rd ed., Academic Press Inc, San Diego, CA, USA, p. 62-141.
- HAMRE K, CHRISTIANSEN R, WAAGBO R, MAAGE A, TORSTENSEN BE, LYGREN B, LIE O, WATHNE E AND ALBREKTSEN S. 2004. Antioxidant vitamins, minerals and lipid levels in diets for Atlantic salmon (*Salmo salar*, L.): effects on growth performance and fillet quality. Aquacult Nutr 10: 113-123.
- HAMRE K, WAAGBO R, BERGE RK AND LIE O. 1997. Vitamins C and E Interact in juvenile Atlantic salmon (*Salmo salar*, L.). Free Radical Biology & Medicine 22(1/2): 137-149.
- HARDIE LJ, FLETCHER TC AND SECOMBES CJ. 1990. The Effect of Viamin E on the Immune Response of the Atlantic Salmon (*Salmo salar* L.). Aquaculture 87: 1-13.
- JARAMILLO Jr F, PENG L AND GATLIN III DM. 2009. Selenium nutrition of hybrid striped bass (*Morone chrysops* x *M. saxatilis*) bioavailability, toxicity and interaction with vitamin E. Aquacult Nutr 15: 160-165.
- JOMORI RK, CARNEIRO DJ, MARTINS MIEG AND PORTELLA MC. 2005. Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems. Aquaculture 243: 175-183.
- KIRON V, PUANGKAEW J, ISHIZAKA K, SATOH S AND WATANABE T. 2004. Antioxidant status and nonspecific immune responses in rainbow trout (*Oncorhynchus mykiss*) fed two levels of vitamin E along with three lipid sources. Aquaculture 234: 361-379.
- LANDOLT ML. 1989. The Relationship Between Diet and the Immune Response of Fish. Aquaculture 79: 193-206.
- LIM C, YILDIRIM-AKSOY M, WELKER T AND KLESIUS PH. 2010. Growth Performance, Immune Response, and Resistance to *Streptococcus iniae* of Nile Tilapia, *Oreochromis niloticus*, Fed Diets Containing Various Levels of Vitamins C and E. J World Aquacult Soc 41(1): 35-48.
- LIN YH AND SHIAU SY. 2005. Dietary vitamin E requirement of grouper, *Epinephelus malabaricus*, at two lipid levels, and their effects on immune responses. Aquaculture 248: 235-244.
- LYGREN B, HJELTNES B AND WAAGBO R. 2001. Immune response and disease resistance in Atlantic salmon (*Salmo salar* L.) fed three levels of dietary vitamin E and the effect of vaccination on the liver status of antioxidant vitamins. Aquacult Int 9: 401-411.
- MARTINS ML, CASTAGNOLLI N, ZUIM SMF AND URBINATI EC. 1995. Influência de diferentes níveis de vitamina C na ração sobre parâmetros hematológicos de alevinos de *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae). R Bras Zootec 12(3): 609-618.
- MONTERO D, MARRERO M, IZQUIERDO MS, ROBAINA L, VERGARA JM AND TORT L. 1999. Effects of vitamin E and C dietary supplementation on some immune parameters of gilthead seabream (*Sparus aurata*) juveniles subjected to crowding stress. Aquaculture 171: 269-278.
- NATT MP AND HERRICK CA. 1952. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci 31: 735-738.

- NAYLOR R AND BURKE M. 2005. Aquaculture and Ocean Resources: Raising Tigers of the Sea. Ann Rev Environ Resour 30:185-218.
- NOROUZITALLAB P, FARHANGI M, BABAPOUR M, RAHIMI R, SINHA AK AND BARUAH K. 2009. Comparing the efficacy of dietary α-tocopherol with that of DL-α-tocopheryl acetate, both either alone or in combination with ascorbic acid, on growth and stress resistance of angelfish, *Pterophylum scalare*, juveniles. Aquacult Int 17: 207-216.
- NRC NATIONAL RESEARCH COUNCIL 2011. Nutrient Requirement of Fish and Shrimp. Washington: National Academic Press, Washington, 105 p.
- PEARCE J, HARRIS JE AND DAVIES SJ. 2003. The effect of vitamin E on the serum complement activity of rainbow trout, *Onchorynchus mykiss* (Walbaum). Aquacult Nutr 9: 337-340.
- PENG LI AND GATLIN III DM. 2009. Dietary vitamin E requirement of the red drum *Sciaenops ocellatus*. Aquacult Nutr 15: 313-319.
- POSTON HA, COMBS Jr GF AND LEIBOVITZ L. 1976. Vitamin E and Selenium Interrelation in the Diet of Atlantic Salmon (*Salmo salar*): Gross, Histological and Biochemical Deficiency Signs. J Nutr 106: 892-904.
- PULSFORD AL, CRAMPE M, LANGSTON A AND GLYNN PJ. 1995. Modulatory effects of disease, stress, copper, TBT and vitamin E on the immune system of flatfish. Fish Shellfish Immun 5: 631-643.
- RANZANI-PAIVA MJT, SALLES FA, EIRAS JC, EIRAS AC, ISHIKAWA CM AND ALEXANDRINO AC. 1998/1999. Análises hematológicas de curimbatá (*Prochilodus scrofa*), pacu (*Piaractus mesopotamicus*) e tambaqui (*Colossoma macropomum*) das estações de piscicultura do Instituto de Pesca, Estado de São Paulo. Bol Inst Pesca 25: 77-83.
- ROSENFELD G. 1947. Corante pancrômico para hematologia e citologia clínica. Nova combinação dos componentes do May-Grünwald e do Giemsa num só corante de emprego rápido. Mem Inst Butantan 20: 329-334.
- SADO RY, BICUDO AJA AND CYRINO JEP. 2008. Feeding Dietary Mannan Oligosaccharides to Juvenile Nile Tilapia Oreochromis niloticus, Has No Effect on Hematological Parameters and Showed Decreased Feed Consumption. J World Aquacult Soc 39(6): 821-826.
- SADO RY, BICUDO AJA AND CYRINO JEP. 2010. Dietary Levamisole Influenced Hematological Parameters of Juvenile Pacu, *Piaractus mesopotamicus* (Holmberg 1887). J World Aquacult Soc 41(S1): 66-75.
- SADO RY AND MATUSHIMA ER. 2008. Histopathological, Immunohistochemical and Ultraestructural Evaluation of Inflammatory Response in *Arius* genus Fish under Experimental Inoculation of BCG. Braz Arch Biol Technol 51(5): 929-935.
- SAU SK, PAUL BN, MOHANTA KN AND MOHANTY SN. 2004. Dietary vitamin E requirement, fish performance and carcass composition of rohu (*Labeo rohita*) fry. Aquacult 240: 359-368.

- SITJÀ-BOBADILLA A AND PÉREZ-SÁNCHEZ J. 1999. Diet related changes in non specific immune response of European sea bass (*Dicentrarchus labrax* L.). Fish Shellfish Immun 9: 637-640.
- STEEL RGD AND TORRIE JH. 1980. Principles and Procedures of Statistics: A biometrical approach. 2nd ed., McGraw-Hill, New York, NY, USA, 569 p.
- TACON AGJ. 1990. Standard Methods for the Nutrition of Farmed Fish and Shrimp. Argent Laboratories Press, Seattle, WA, USA, 208 p.
- TAVARES-DIAS M, MARTINS ML, SCHALCH SHC, ONAKA EM, QUINTANA CIF, MORAES JRE AND MORAES FR. 2002. Alterações hematológicas e histopatológicas em pacu, *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae), tratado com sulfato de cobre (CuSO₄). Acta Sci 24(2): 574-554.
- TAVARES-DIAS M AND MATAQUEIRO MI. 2004. Características hematológicas, bioquímicas e biométricas de *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) oriundos de cultivo intensivo. Acta Sci Biol Sci 26(2): 157-162.
- TAVARES-DIAS M, TENANI RA, GIOLI LD AND FAUSTINO CD. 1999. Caracterísitcas hematológicas de teleósteos brasileiros.
 II. Parâmetros sangüíneos do *Piaractus mesopotamicus* Holmberg, 1887 (Osteichthyes: Characidae) em policultivo intensivo. Rev Bras Zool 16: 423-431.
- THORARINSSON R, LANDOLT MA, ELLIOTT DG, PASCHO RJ AND HARDY RW. 1994. Effect of dietary vitamin E and selenium on growth, survival and the prevalence of *Renibacterium salmoninarum* infection in chinook salmon (*Oncorhynchus tshawytscha*) Aquaculture 121: 343-358.
- TOCHER DR, MOURENTE G, VAN DER EECHEN A, EVJEMO JO, DIAZ E, WILLE M, BELL JG AND OLSEN Y. 2003. Comparative study of antioxidant defence mechanisms in marine fish fed variable levels of oxidized oil and vitamin E. Aquacult Int 11: 195-216.
- TRENZATO CE, HIGUERA M AND MORALES AE. 2007. Influence of dietary vitamins E and C and HUFA on rainbow trout (*Oncorhynchus mykiss*) performance under crowding conditions. Aquaculture 236: 249-258.
- TRUSHENSKI JT AND KOHLER CC. 2008. Influence of Vitamin E Source and Dietary Supplementation Level on Production Performance of Sunshine Bass, *Morone chrysops*♀ x *Morone saxatilis*♂, Fillet Tocopherol Content, and Immunocompetency during Stress and Bacterial Challenge. J World Aquacult Soc 34(4): 454-466.
- URBINATI EC AND GONÇALVES FD. 2005. Pacu (*Piaractus mesopotamicus*). In: BALDISSEROTTO B AND GOMES LC (Eds), Espécies nativas para piscicultura no Brasil. Editora UFSM, Santa Maria, RS, Brasil, p. 225-255.
- WINTROBE MM. 1934. Variations on the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematol 51: 32-49.
- WISE DJ, TOMASSO JR, SCHWEDLER TE, BLAZER VS AND GATLIN III DM. 1993. Effect of vitamin E on the immune responses of channel catfish to *Edwardsiella ictaluri*. J Aquat Anim Health 5:183-188.