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SHORT NOTE

Evaluation of the Phytase Enzyme in Granulated and Liquid Forms for Nile Tilapia *(Oreochromis niloticus)*

Pontes, T.C.[1@], França, W.G.[2], Dutra, F.M.[3], Portz, L.[4] and Ballester, E.L.C.[3,4]

[1]Programa de Pós-graduação em Zootecnia. Universidade Estadual de Maringá (UEM). Maringá. Paraná. Brasil.
[2]Programa de Pós-graduação em Ciência Animal e Pastagens. Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP). Piracicaba. São Paulo. Brasil.
[3]Laboratório de Carcinicultura. Programa de Pós-Graduação em Aquicultura e Desenvolvimento Sustentável. Universidade Federal do Paraná, Setor Palotina (UFPR-Palotina). Palotina. Paraná. Brasil.
[4]Departamento de Zootecnia. Programa de Pós-Graduação em Aquicultura e Desenvolvimento Sustentável. Universidade Federal do Paraná, Setor Palotina (UFPR-Palotina). Palotina. Paraná. Brasil.

Additional keywords

Nutrition.
Phosphorus.
Phytate.
Inositol hexaphosphate.
Feed formulation.

SUMMARY

The aim of the study was to evaluate the zootechnical performance of Nile tilapia (Oreochromis niloticus) fed with plant ingredients, supplemented with granulated and liquid phytase enzyme. To carry out the study was used 600 Nile tilapia juveniles (mean initial weight of 1.72 ± 2.21 g and mean initial length of 9.46 ± 0.64 cm) sexually inverted into males, during 64 days. The experimental design was completely randomized with six diets: negative control (without inorganic phosphate and without addition of phytase), positive control (with inorganic phosphate), Two diets (1500 and 3000 FTU/kg) with phytase in the granulated and two diets (1500 and 3000 FTU/kg) with phytase in liquid, in with five replicates. The diets were isoenergetic with 3100 kcal/kg and isoproteic with 28% crude protein. The granulated phytase was added before and after extrusion and the liquid phytase added after processing. Phytase liquid form provided best performance and the best protein content in the carcass. The same also was observed for the coefficients of apparent digestibility of protein and mineral matter. Retention of phosphorus in the plasma and vertebrae was not influenced by the treatments. With the results it is concluded that the addition of phytase in the liquid form is more efficient, and the diets can be added at lower levels.

Palavras chave adicionais

Nutrição.
Fósforo.
Fitato.
Hexafosfato de inositol.
Formulação de alimento.

Avaliação da Enzima de Fitase em Formas Granuladas e Líquidas para Tilapia do Nilo *(Oreochromis niloticus)*

RESUMO

O objetivo do estudo foi avaliar o desempenho zootécnico da tilápia do Nilo (Oreochromis niloticus) alimentado com ingredientes vegetais, complementado com enzima fitase granulada e líquida. Para realizar do estudo, utilizou-se 600 juvenis de tilápia do Nilo (peso inicial médio de 1,72 ± 2,21 g e comprimento inicial médio de 9,46 ± 0,64 cm) invertidos sexualmente para machos, durante 64 dias. O design experimental foi inteiramente casualizado com seis dietas: controle negativo (sem fosfato inorgânico e sem adição de fitase), controle positivo (com fosfato inorgânico), duas dietas (1500 e 3000 FTU/kg) com fitase granuladas e duas dietas (1500 e 3000 FTU/kg) com fitase líquida, com cinco repetições. As dietas foram isoenergéticas com 3100 kcal/kg e isoproteicas com 28% de proteína bruta. A fitase granulada foi adicionada antes e depois da extrusão e a fitase líquida adicionada após o processamento. A forma líquida de fitase forneceu o melhor desempenho e o melhor conteúdo protéico na carcaça. Também foi observado o mesmo quanto aos coeficientes de digestibilidade aparente de proteína e matéria mineral. A retenção de fósforo no plasma e nas vértebras não foi influenciada pelos tratamentos. Com os resultados concluiu-se que a adição de fitase na forma líquida é mais eficiente, podendo ser adicionadas nas dietas níveis mais baixos.

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taniacristina.bio@gmail.com

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) stands out in continental aquaculture for its desirable characteristics such as easy management, resistance to environmental changes, high acceptance in the consumer market (Barros *et al.*, 2016), and fast growth rate, besides allowing high percentages of plant origin ingredients used in their feed formulation (Furuya, 2010).

In ingredients of plant origin, phosphorus (P) occurs mainly in the complexed form of phytate (inositol hexaphosphate) little available for monogastric animals since they do not synthesize the phytase enzyme responsible for breaking down phytate and making P and other nutrients available (Mota *et al.*, 2014). Thus,

one of the main issues limiting the use of ingredients of plant origin in fish nutrition is the presence of phytate (CAO *et al*., 2007), which, besides having low availability, may also be considered an antinutritional factor as it acts as a chelating agent for several bivalent cations among which calcium, iron, magnesium, zinc (Surek *et al*., 2008) and the protein fraction of the food to form insoluble complexes and decreas the feed's metabolizable energy due to the negative influence on nutrient digestion (Ludke *et al*., 2001).

The use of phytase in feed containing ingredients of plant origin is the subject of several studies aiming to increase nutrient availability and decrease P and N excretion into the aquatic environment (Bock *et al*., 2007). Phytase activity is expressed as FTU (active phytase unit), being 1 FTU the amount of inorganic phosphorus released (µmol) over 1 min of reaction in a sodium phytase solution at 5.1 mmol.L^{-1}, pH 5.5, and 37 °C (Engelen *et al*., 1994).

When adding phytase to feed, it must undergo proper handling due to its instability at high temperatures, i.e., the thermal treatment may destroy its effect. The enzyme may be added to fish feed in powder, granulated, or liquid forms either before or after processing. Post-processing addition may prevent thermostability problems at high coating temperatures (>80 °C) (Cao *et al*., 2007).

The aim was to evaluate the zootechnical performance of Nile tilapia (*O. niloticus*) fed with plant ingredients, supplemented with different levels (1500 and 3000 FTU/kg^{-1}) granulated and liquid phytase enzyme (BASF®).

MATERIAL AND METHODS

The study was carried during 79 days, with 64 days for performance evaluation and 15 days for digestibility evaluation. The research used 600 Nile tilapia (*O. niloticus*) juveniles sexually inverted into males with mean initial weight of 11.72±2.21 g and mean initial length of 9.46±0.64 cm. The fish were distributed in six treatments completely randomized (100 juveniles each) and five repetitions. The experimental units were 30 polyethylene boxes with volume of 300 L (20 juveniles per box) in a recirculation system with biological filtering, individual aeration and natural photoperiod.

The feeds were ground and sieve on a 1.5 mm, homogenized and mixed (Y mixer) with the components, added with 0.1% of inert indicator Cr_2O_3, moistened with 20% distilled water, and extruded (4.0 mm) with different levels and forms of phytase (Natuphos; BASF®) **(Table I)**.

The experimental diets used in this study were isoenergetic at 3,100 kcal/kg of digestible energy (DE)

Table I. Ingredients and nutritional levels analyzed in the feeds. Values expressed as 100% of original matter
(Ingredientes y niveles nutricionales analizados en los piensos. Valores expresados como 100% de la materia original).

Ingredients	Phytase Levels (FTU/kg)					
	NC Negative Control	PC Positive Control	GP 1,500 FTU/kg^{-1} Granulated	GP 3,000 FTU/kg^{-1} Granulated	LP 1,500 FTU/kg^{-1} Liquid	LP 3,000 FTU/kg^{-1} Liquid
Corn meal	46.6	46.6	46.6	46.6	46.6	46.6
Soybean meal	46.6	46.6	46.6	46.6	46.6	46.6
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Premix®[a]	1.00	1.00	1.00	1.00	1.00	1.00
$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00
NaCl	0.40	0.40	0.40	0.40	0.40	0.40
Cr_2O_3	0.10	0.10	0.10	0.10	0.10	0.10
Corn starch	2.00	0.00	1.97	1.95	1.99	1.97
Photoset	0.00	0.00	0.03	0.05	0.01	0.03
NaH_2PO_4	0.00	2.00	0.00	0.00	0.00	0.00
Cr_2O_3[b]	0.01	0.01	0.01	0.01	0.01	0.01
Nutrient composition						
Crude protein	28.17	28.17	28.17	28.17	28.17	28.17
Ethereal extract	6.07	6.07	6.07	6.07	6.07	6.07
Moisture	7.97	9.48	8.05	8.93	8.32	7.97
Mineral Matter	5.43	5.43	5.43	5.43	5.43	5.43
Calcium	0.58	0.62	0.58	0.58	0.58	0.58
Phosphorus	0.48	0.89	0.43	0.39	0.41	0.39

NC – Negative control (no inorganic phosphate), PC – Positive control (with inorganic phosphate), GP – 0.03% (1,500 FTU/kg^{-1}) granulated, GP – 0.05% (3,000 FTU/kg^{-1}) granulated, LP – 0.01% (1,500 FTU/kg^{-1}) liquid, and LP – 0.03% (3,000 FTU/kg^{-1}) liquid. [a] Guaranteed levels per kg of the product: Vit. A, 1,750,000 UI; Vit. D3, 375,000 UI; Vit. E, 20,000 UI; Vit. K3, 500 mg; Vit. B1, 2,000 mg; Vit. B2, 2,500 mg; Vit. B6, 2,500 mg; Vit. B12, 5,000 mg; Folic acid, 625 mg; Ca pantothenate, 7,500 mg; Vit. C, 37,500 mg; Biotin, 50 mg; Inositol, 12,500 mg; Niacin, 8,750 mg; Choline, 100,000 mg; Co, 50 mg; Cu, 1,250 mg; Fe, 15,000 mg; I, 100 mg; Mn, 3,750 mg; Se, 75 mg; Zn, 17,500 mg. bCr2O3 – chromium oxide.

and isoproteic at 28% crude protein (Boscolo *et al*., 2006). The diets underwent bromatological analyses to determine dry matter, mineral matter, ethereal extract, and crude protein. The enzyme activity of phytase in the feeds was analyzed according to the methodology described by Engelen *et al*. (1994) using HPLC and atomic absorption spectrophotometry in CBO laboratory tests, Valinhos, SP, Brazil. The phytase (Natuphos; BASF®) added to the feed had concentration of 5,000 FTU/kg^{-1} for granulated phytase and 10,000 FTU/kg^{-1} for liquid phytase. The granulated form was added to the ingredient mix at 1,500 FTU/kg^{-1} and 3,000 FTU/kg^{-1}.

The inclusion of the enzyme in the granulated form occurred during the mixing of the ingredients, then this was homogenized and extruded at a temperature of approximately 180 ° C. As the feed passed through the processing the outlet temperature was measured to be 75°C.

Liquid phytase was added after the granules were extruded and dried. Phytase was diluted in 4 mL water per kg of feed and added by spraying with a manual pump according to Silva *et al*. (2007) at 1,500 FTU/kg and 3,000 FTU/kg. The fish were fed two daily meals at 8 a.m. and 5 p.m. until apparent satiation.

The water varaible were monitored for both the performance evaluation and the digestibility evaluation. Water temperature was measured twice a day using a digital thermometer (ICOTERM®; model 6132), oxygen was measured daily using an oximeter (ALFAKIT®; model AT 170), and pH was determined weekly using a portable digital pH meter (ALFAKIT®; model AT 315). The analyses of ammonia, nitrite, alkalinity, and hardness were performed weekly according to the methodologies by APHA (1980). The tanks were siphoned weekly as needed.

At the beginning and end of the experiment, the fish were weighed to determine weight gain (WG) = final weight - initial weight; feed intake = total feed given – wastes, fixed by mortality, apparent feed conversion (AFC) = feed intake/weight gain; protein efficiency rate (PER) = 100 x (weight gain/amount of protein consumed); specific growth rate (SGR) = ((lnFinal Weight – lnInitial Weight) x 100)/days total; survival (S) = 100 x (initial no. of fish - final no. of fish)/initial no. of fish.

For the final weighing, the animals were anesthetized with Eugenol® (clove oil solution to 150 mg.L^{-1}) at 150 mg.L^{-1} (Simões *et a*l. 2012). Next, 25 fish from each treatment were sacrificed for analysis of the chemical composition of the carcass according to the methodology by the AOAC (2000).

Blood plasma analysis was performed at the end of the performance experiment. For analysis were used 15 fish, where three fish were withdrawn from each replicate corresponding to the treatment. the fish were kept in fasting for 12 h and the blood was collected in 1 mL^{-1} syringes with heparinized needles (0.60 x 25 mm). Five hundred μl^{-1} of blood were collected per puncture of the caudal vein of three fish from each experimental unit. After collection, the blood was centrifuged at 13,000 rpm for 10 min to separate the plasma and then stored at -20 °C. The plasma phosphorus analysis was performed by spectrophotometry in an automated biochemical analyzer (MINDRAY BS 120) using a commercial UV phosphorus kit (Biotécnica®).

For phosphorus retention analyzes the 15 fish that had been used in the blood analysis were used. The analysis was performed by the metavanadate calorimetry method by nitric perchloric digestion according to the methodology by Institute Adolfo Lutz (2008).

For the digestibility study, 60 juveniles of Nile tilapia (12 ± 0.1g) were obtained from fish farms in the region. These were distributed in three rigid 80 L cages with screen bottoms (20 fish per cage), kept in polypropylene tank with a capacity of 1000 L. The temperature and the dissolved oxygen of the aquaria water of feed and digestibility were maintained by means of aerator, heater and thermostat and partial water exchange. The food was offered during the day at 13, 15 and 17h. Prior to sampling, the fish were fed a reference diet for seven days. To avoid mixing feces between diets in each collection, the feces of the first three days were excluded. During the night the fish were transferred to fecal collection aquariums (200L), also individual to the treatments, where they remained until the morning of the following day. The faeces were collected by the modified Guelph method (Pezzato et al., 2002). Sampling were done in triplicate, being considered as repetition the set of feces collected in each aquarium. The fecal collection for each diet was 15 days. After collected through sedimentation, centrifuged at 4 °C and stored in a freezer at -10 °C. Chromic oxide was determined according to the procedure described by (Furukawa and Tsukahara, 1966). The ADCs (%) (Maynard and Loosly, 1969) of protein and ashes of the experimental diets were calculated according to De Silva and Anderson (1995), as follows: ADC =100[1-(Cr_2O_3 diet/Cr_2O_3 feces) (feces nutrient or energy/diet nutrient or energy)]

The data were submitted to the normality test by the Kolmogorov-Smirnov and Liliefors test and the Levene test was checked for homogeneity. When these two requirements were met, the results were subjected to analysis of variance and Tukey's test (α=0.05) to determine statistical differences among the treatments.

RESULTS AND DISCUSSION

There was no difference (P>0.05) for the water quality variables among treatments. The concentrations of dissolved oxygen were 7.42 ± 0.56 and 26.07 ± 0.4; temperature of 27.04 ± 1.76 and 26.07 ± 0.4; pH of 7.36 ± 0.63 and 6.03 ± 0.6; total ammonia <0.05 and nitrite <0.08, respectively for the test of zootechnical performance (64 days) and digestibility (15 days). The water quality values observed during the experimental period were within the recommended range for the species (Sipaúba-Tavares, 1995).

The activity of the enzyme in granulated and liquid forms added before and after processing differed (P<0.05). Treatments negative control (NC), positive control (PC), and granulated phytase (GP) GP-1,500 FTU/kg^{-1} had active phytase activity below 100

FTU/kg^{-1}. Treatment GP-3,000 FTU/kg^{-1} also showed a reduction in enzyme activity due to the thermal treatment, however, the activity was higher compared to treatment GP-1,500 FTU/kg^{-1}. Treatments liquid phytase (LP) LP-1,500 FTU/kg^{-1} and LP-3,000 FTU/kg^{-1} showed no reduction in enzyme activity. In the present study, the high temperature during thermal processing reduced the activity of phytase in treatments GP-1,500 and GP-3,000 FTU/kg^{-1} when granulated phytase added prior to extrusion. According to Jermutus *et al*. (2001), microbial phytase is stable in a broad temperature range and its maximum activity is close to 60 °C. Extrusion temperature in the study was 105 °C, which justifies enzyme denaturation. Naves *et al*. (2012), when assessing activity of phytase of different origins (*Aspergillus oryzae*, *Aspergillus niger*, and *Saccharomyces cerevisae*) at different temperatures and pH, observed the inactivation of the three enzymes at over 60 °C. Microbial phytases are sensitive to high temperatures due to the influence they have on the activity and conservation of structures and biomolecules (Gomes et al., 2007). Temperature above 50-55 ° C results in structural modifications, reducing the catalytic power of the enzymes (Marzzoco, 2007). The added granulated phytase prior to processing may have its activity reduced due to high temperature which does not occur with the inclusion of phytase in the liquid form added after the extrusion process and may be added at lower levels.

Survival was not impacted by phytase supplementation in the diet. Dietary supplementation with phytase did not impact the survival rate of Nile tilapia juveniles, proving the supplementation levels employed had no negative effect on this variable. A similar result for Nile tilapia was observed by Bock *et al*. (2006).

The parameters of final weight, final length, weight gain, specific growth rate, protein efficiency rate, and apparent dietary conversion in the PC treatment with inorganic phosphorus addition differed (P<0.05) compared to the other treatments. Treatment GP-1,500 FTU/kg^{-1} had the lowest results for the parameters assessed. For treatments NC, GP-3,000, LP-1,500, and LP-3,000 FTU/kg^{-1}, the parameters of final weight, mean weight gain, specific growth rate, and protein efficiency rate did not differ (P>0.05) (**Table II**).

Table II. Mean Values (±S.D.) of productive performance, digestibility, centesimal composition of the carcasses based on dry matter and phosphorus concentration in the plasma and vertebrae of Nile tilapia (Oreochromis niloticus) subjected to an isoenergetic (3,100 kcal/kg-1 DE) and isoproteic (28% CP) diet added with different levels of granulated and liquid phytase (Valores medios (± D.E.) de rendimiento productivo, digestibilidad, composición centesimal de las canales basados en materia seca y concentración de fósforo en el plasma y vértebras de la tilapia del Nilo (Oreochromis niloticus) sometidos a un isoenergético (3.100 kcal /kg-1 DE) y dieta isoproteica (28% CP) añadida con diferentes niveles de fitasa granulada y líquida.)

Parameters	Granulated and liquid phytase values (FTU/kg^{-1})					
	NC	PC	GP		LP	
	Negative Control	Positive Control	1500 (FTU/kg^{-1})	3000 (FTU/kg^{-1})	1500 (FTU/kg^{-1})	3000 (FTU/kg^{-1})
FW (g)	34.8 ± 2.7^{ab}	42.5 ± 3.5^{a}	32.8 ± 1.9^{b}	35.8 ± 7.4^{ab}	35.9 ± 3.9^{ab}	35.3 ± 3.5^{ab}
FL (cm)	12.3 ± 0.2^{b}	13.3 ± 0.3^{a}	12.4 ± 0.2^{b}	12.4 ± 0.7^{b}	12.5 ± 0.4^{ab}	12.4 ± 0.2^{b}
WG (g)	23.1 ± 2.7^{ab}	30.8 ± 3.5^{a}	21.0 ± 1.9^{b}	24.0 ± 7.4^{ab}	23.5 ± 3.9^{ab}	23.6 ± 3.5^{ab}
SGR (%)	1.70 ± 0.12^{ab}	2.00 ± 0.12^{a}	1.6 ± 0.05^{b}	1.7 ± 0.31^{ab}	1.7 ± 0.17^{ab}	1.7 ± 0.15^{ab}
PER (%)	8.90 ± 1.0^{ab}	11.90 ± 1.4^{a}	8.1 ± 0.5^{b}	9.7 ± 2.9^{ab}	9.0 ± 1.5^{ab}	9.1 ± 1.40^{ab}
AFC (g/g)	1.30 ± 0.1^{ab}	1.10 ± 0.04^{a}	1.6 ± 0.3^{b}	1.7 ± 0.4^{b}	1.4 ± 0.2^{ab}	1.3 ± 0.06^{ab}
S (%)	83 ± 2.1	98 ± 0.5	92 ± 1.2	90 ± 5.5	94 ± 0.2	95 ± 0.8
ADC_p (%)	44.1 ± 0.7^{b}	82.5 ± 1.5^{a}	32.8 ± 1.9^{b}	85.8 ± 1.4^{a}	85.9 ± 0.9^{ab}	79.3 ± 0.5^{ab}
ADC_{mm} (%)	56.1 ± 0.8^{b}	73.3 ± 0.3^{a}	42.4 ± 0.2^{b}	72.4 ± 0.7^{a}	73.5 ± 0.4^{a}	71.4 ± 0.2^{b}
Centesimal composition of the carcasses based on dry matter						
C. P. (%)*	52.63±3.73^{b}	65.23± 2.25^{a}	62.14±6.13^{a}	58.26±4.97^{ab}	65.42±6.06^{a}	67.28±3.95^{a}
E. E (%)**	22.29±1.67^{b}	23.58±2.89^{ab}	24.79±2.59^{ab}	26.98±2.78^{a}	25.99±1.87^{ab}	25.26±2.12^{ab}
Ashes (%)	9.95±0.59^{c}	14.82±1.45^{a}	11.67±0.71^{bc}	11.35±1.76^{bc}	12.14±0.69^{bc}	12.74±1.19^{ab}
Moisture (%)	69.34±4.68^{b}	73.98±1.76^{a}	73.59±2.03^{a}	71.30±5.83^{ab}	73.22±2.34^{a}	74.25±1.28^{a}
Phosphorus concentration in the plasma and vertebrae						
P.P. (mg/dL)•	16.5±1.9^{b}	18.7±3.2^{b}	17.4±3.5^{b}	26.8±2.6^{a}	28.1±4.1^{a}	25.4 ±1.6^{a}
V.P. (%)•	0.61±0.05^{b}	0.90±0.03^{a}	0.64±0.03^{ab}	0.76±0.07^{ab}	0.81±0.18^{ab}	0.88±0.01^{ab}

Mean values compared by Tukey's test (p<0.05) regarding final weight (FW), final length (FL), protein efficiency rate (PER), specific growth rate (SGR), weight gain (WG), apparent feed conversion (AFC), survival (S), and apparent digestibility coefficient of protein (ADCp) and mineral matter (ADCmm). * Crude protein; ** Ethereal extract; • Plasma phosphorus and • Vertebrae phosphorus.

Adding granulated phytase prior to processing had no positive impact on productive performance due to the reduction in enzyme activity. The reduction in performance is related to the lower protein digestibility coefficient, with the denaturation of the enzyme, the processing of the nutrients that may have unavailable the mirerais important for the performance. Low levels of available phosphorus may have influenced performance results. Brandão (2011) and Godoy et al. (2016) report that the reduction in performance can be attributed to the insufficient use of phosphorus in the diet. For Bock et al. (2007) and Costa et al. (2013) in studies with Nile tilapia fingerlings found that diets with low levels of available phosphorus restricted the use of dietary protein, thus affecting animal performance. Adding the enzyme in liquid form is an alternative that has been employed to prevent the loss of phytase activity at high temperatures, particularly in the extrusion process. The enzyme is usually added by mixing it concentrated with a stabilizing agent and then spraying it onto the extruded or pelletized diet (Kumar et al., 2011).

For feed conversion effects, the best results were observed in the positive control with supplementation of inorganic phosphorus (highly absorbable) followed by treatments LP-1,500 and 3,000 FTU/kg^{-1}. With alternative inclusion of granulated phytase, similar results were observed by Bock *et al*. (2007) for Nile tilapia when using phytase concentrations of 1,000, 1,500, and 2,000 FTU/kg^{-1} dissolved in distilled water and sprayed after pelletizing. Brandão (2011) also did not find significant results in the performance of tambaquis (*Colossoma macropomum*), using the levels of 1,000, 1500 and 2000 FTU / kg-1 supplemented in extruded diets. Those authors found no positive results for weight gain or feed conversion of the fish receiving feed containing phytase. The results showed that despite the inclusion of phytase in liquid form at high levels, presented lower results when compared to the positive control, this fact may be related to the degree of grinding of the food that may influence the mineral use and nutrient solubility at the point of contact with the absorptive membrane (Li et al., 1996).

Dietary supplementation with phytase in the present experiment impacted the apparent digestibility coefficient of Nile tilapia juveniles, confirming that the supplementation levels used had an effect on this variable, which matches the reports by Bock *et al*. (2006) for Nile tilapia. From the results observed, the best apparent digestibility coefficients of protein and mineral matter were determined for PC, NC, GP-3,000 FTU/kg^{-1}, and LP-1,500 FTU/kg^{-1}.

Phosphorus (P) retention in the plasma or the vertebrae showed no significant results (P>0.05) (**Table II**). Vielma et al. (1998), when assessing the effect of liquid phytase at 1,500 FTU/kg-1 added after pelletizing on blood plasma of *Oncorhynchus mykiss*, observed an increase in plasma phosphorus concentration. The results of the present study are in accordance with Ferreira (2011), who found no increase in plasma phosphorus concentration of *Rhamdia quelen* when granulated phytase was added at 1,500 FTU/kg-1 prior to processing. Plasma phosphorus results may be related to low availability of this mineral, which even with the inclusion of the enzyme may be present in inadequate amounts. For Lall (2002), low phosphorus intake leads to low absorption of this mineral, thus reducing phosphorus levels in the plasma.

The results of carcass centesimal composition are presented in **Table II**. Significant differences were observed (P<0.05) for carcass protein and moisture. In treatment GP-1,500 FTU/kg^{-1}, phytase activity dropped, however, protein retention in the carcass was higher compared with treatment GP-3,000 FTU/kg^{-1}, which had higher enzyme activity after extrusion. A significant difference (P<0.05) was also observed in the deposition of ethereal extract in the carcass in relation to treatment NC and the other treatments. Carcass ashes showed statistical differences (P<0.05) between treatments PC and LP-3,000 FTU/kg^{-1}, while the other treatments did not differ among themselves.

The carcass composition results in the present study showed an increase in protein retention in the carcass of fish fed diets containing liquid phytase, a value close to that found in the positive control treatment with the inclusion of inorganic phosphorus. The increase in protein retention in the carcass may be justified by the reduction of the phytate protein complex, which increases nutrient availability (Liebert and Portz, 2005).

The amount of ashes increased in the fish's whole body. The liquid form at 3,000 FTU/kg^{-1} proved more effective, similar to the positive control treatment (supplemented with inorganic P). The treatments supplemented with granulated enzyme were insufficient to increase the ash content in the carcasses. For Hung *et al*. (2015), the concentration of 1,500 FTU/kg^{-1} added prior to pelletizing was sufficient to increase ash concentration in the whole body of *Pangasianodon hypophthalmus*. Silva *et al*. (2007), when assessing the effect of liquid phytase added after extrusion at 250, 500, and 1,000 FTU/kg^{-1}, found no effect of phytase on the contents of moisture, crude protein, or ashes in the carcass of Nile tilapia.

The deposition of lipids decreased in the carcass of fish fed the negative and positive control diets, granulated phytase at 1,500 FTU/kg-1, and in the treatments with addition of liquid phytase. The lower concentration of lipids in the carcasses of fish fed the negative control treatment may be associated with the presence of native phytase, which, even with phosphorus deficiency, may have provided this mineral through phytate hydrolysis. Ferreira (2011) observed a reduction in body fat accumulation with the inclusion of granulated phytase at 1,500 FTU/kg-1 added prior to processing, however, no differences were found in the percentages of dry matter, protein, or ashes with the inclusion of the enzyme for juveniles of *R. quelen*. The high accumulation of lipids in the whole body of fish fed treatment four may be related to the deficiency in phosphorus of that treatment due to enzyme denaturation during extrusion. Lall (2002) reports that phosphorus deficiency in the diet may cause inhibition of β-oxidation of fatty acids, which increases the accumulation of lipids in the body.

CONCLUSIONS

The positive control diet with the addition of inorganic phosphorus showed the best performance

results, followed by the inclusion of phytase in the granular form at the 3000 FTU/kg^{-1} level and by 1500 and 3000 FTU / kg^{-1} levels in the liquid form. Liquid phytase added after extrusion is more efficient than the granulated form added prior to processing and may be added at lower levels. Phosphorus retention in the plasma and vertebrae was not influenced by the treatments.

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