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Improving nutrient availability of defatted rice bran using different phytase sources applied to grass carp (*Ctenopharyngodon idella*) diet

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Abstract: In the present study, we evaluated the effects of the hydrolysis of phytate of defatted rice bran (DRB) by a pretreatment with non-commercial phytase produced by *Saccharomyces cerevisiae* (DRB-PS) compared to the application of Natuphos® (commercial phytase produced by the BASF Company) (DRB-PN) in diets for grass carp, *Ctenopharyngodon idella*. Fish (57.55 ± 0.4 g) fed one of the experimental diets in triplicates for 35 days. Effects of the phytase used on blood parameters, intestinal proteases and hepatic glucose were not observed ($p > 0.05$). Similarly, no differences were found for serum phosphorus (P). However, were found higher levels of calcium (9 and 5.25%) in the control treatment in relation to DRB-PS and DRB-PN respectively, besides higher calcium-phosphorus ratio was found in this treatment. For the fish carcass composition was not statistically different ($p > 0.05$) except total lipids, which showed its highest content in fish fed on the DRB-PN diet ($p < 0.05$). The obtained results suggested that the use of the phytase, irrespective to its source may eliminate the use of traditional P sources in fish diets.

Key words: enzymes, fish, mineral availability, nutrition, phytates, rice bran.

INTRODUCTION

The grass carp (*Ctenopharyngodon idellus*) is as typical herbivorous fish and one of the most important freshwater species cultured in the world (FAO 2018). In its natural environment, it feeds on vegetables present in the water (Du et al. 2005). The dietary products, which have plant origin, are being used in animal feeds including fish. However, plant ingredients contain antinutritional factors, such as phytic acid or phytase, which form complexes with minerals, proteins, lipids, and starches (Francis et al. 2001, Graf & Faton 1990). These complexes are not absorbed in the gastrointestinal tract of fish, lowering their digestion and bioavailability (Gilani et al. 2005, Greiner & Konietzny 2006, Kumar et al. 2011, Sugiura et al. 2001). However, the presence of phytic acid and other anti-nutritional factors in plants feedstuffs has been a major obstacle to be used (Storebakken et al. 1998). Most of the minerals found in plant foods are chelated due to their phytate content and become unavailable to monogastric animals (Conte et al. 2002). Thus, inorganic phosphorus, a non-renewable and expensive mineral and important due its participation in several bodily functions, is added to the diets of fish to meet their nutritional needs. The excess of phosphorus added to the feed, phytate-related phosphorus and other unused minerals are

excreted in the feces of the animal, contributing to environmental contamination (Mallin 2000).

It is known that fish do not synthesize phytase; thus, the dephytinization process is necessary due to high and variable phytate content among plant species, which directly affects the phosphorus bioavailability (Conte et al. 2002). Phytase supplementation to fish increases the availability of phosphorus and others nutrients in feed formulated from plant sources (Liu et al. 2013, 2014, Kemigabo et al. 2018). Among the plant foods, rice bran has the highest percentage of total phosphorus (1.5%), while corn and soybean bran levels are 0.28% and 0.65%, respectively (NRC 1994), and it has a lower market price than many other brans thus, its use can lower the final cost of feeds (Moreira et al. 2003). Phytase incorporation in fish diets is suggested as a way of increasing the production of herbivorous carp (Liu et al. 2013, 2014, Kemigabo et al. 2018) especially diets formulated with plants by-products (mixtures) of grains, flours and crop residues (Mukhopadhyay & Kaushik 2001).

Considering the above, this study tried to verify the efficiency of a phytase derived from yeast *(Saccharomyces cerevisiae)* and commercial phytase in improving the availability of phosphorus found in rice bran and to verify the metabolic effects of the use of rice bran in the preparation of feeds for grass carp (*Ctenopharyngodon idella*).

MATERIAL AND METHODS

Production of *S. cerevisiae* pre-inoculum and inoculum

The yeast used in the present study was *S. cerevisiae* strainzi (EU188613) selected as a potential producer of thermostable phytase by Ries & Macedo (2009). The pre-inoculum was prepared by suspending the spores from the solid Yeast Malt (YM) culture in 2.5 mL of sterilized water.

The inoculum was prepared as follows: 3 mL of pre-inoculum were added to 100 mL of a medium consisting of 10 g L^{-1} of yeast extract, 20 g L^{-1} of peptone and 20 g L^{-1} of glucose, and the whole was kept under fermentation for 12 hours in Marconi MA 505 Mini Batch Reactors (Piracicaba, São Paulo, Brazil) with agitation at level 3 and at a temperature of 35 °C.

Enzymatic extract production

The phytase production from *S. cerevisiae* was performed at the Laboratório de Bioquímica de Alimentos, Universidade Estadual de Campinas – UNICAMP, in Marconi MA 505 Mini Batch Reactors (Piracicaba, São Paulo, Brazil) with agitation. The liquid medium consisted of (%): 2.5 Sucrose, 0.5 Sodium phytate, 0.15 urea, 0.05 $MgSO_4.7H_2O$, 0.05 KCl; 0.0001 $FeSO_4.7H_2O$; 0.00075 $MnSO_4.H_2O$, and 0.01 $CaCl_2$. Inoculum was added to a concentration of 10% and the fermentation was carried out in Mini Batch Reactors (Marconi MA 505, Piracicaba, São Paulo, Brazil) with agitation at level 6 for 36 hours at a temperature of 30 °C. After the culture development, the media were centrifuged (Hitachi centrifuge model CR Himac 21GII, Japan) at 7,100 x *g* at a temperature of 4 °C for 30 minutes. The supernatant fraction was the extracellular enzyme extract. The intracellular extract was obtained after ultrasonication (180 - 200 watts), 3 times, for 15 seconds each in Ultrasonic Tip Labsonic Systems (Lab Line Instruments, Dallas, USA) of the cell mass re-suspended in Tris-HCl, pH 7.0, 0.1 M. The fraction extracellular plus the fraction intracellular comprise the total enzyme extract.

Treatment of the defatted rice bran with phytase

A treatment with commercial Natuphos® enzyme (commercial phytase produced by the BASF Company, 100000G) was used for comparison, as was as a control diet was formulated with untreated rice bran supplemented with calcium phosphate. The enzymatic treatment of the rice bran was performed according to a modified version of the method proposed by Storebakken et al. (1998). The rice bran was incubated with enzyme extract in a ratio of 1:3 (w/v), pH 5.5, with agitation to facilitate the enzymatic activity. The enzymatic extract produced by the *S. cerevisiae* strain zi (EU188613) demonstrated an activity of 550 FTU kg^{-1} DRB. The commercial Natuphos® enzyme was used in equivalent activity level and conditions. The mixing was done using a manual mixer for 2 hours in a water bath at 40 °C. After incubation, the mixtures were dried at 55 °C until moisture content was below 12%. Once cooled, the rice bran was milled and used in the feed preparation.

To analyze the phosphorus content of the rice bran, it was first treated with 1.0N HCl (pH 3.0) at a ratio of 1:10 (w/v) for 30 minutes under agitation followed by three successive centrifugations (1,320 x *g* for 5 minutes) to obtain the release of soluble phosphorus (Towo et al. 2006). Insoluble phosphorus in the remaining fraction was determined after acid digestion using a catalytic mixture of copper sulfate and potassium sulfate. The determination of total phosphorus was obtained after the acid digestion of the rice bran and the re-suspension of the extract in distilled water. The quantification of the phosphorus fractions of the bran was determined colorimetrically at 700 nm as proposed by Shimizu (1992) in a Beckman Coulter DU640 Spectrophotometer (Fullerton, CA, USA) and by means of a calibration curve with K_2HPO_4 (ECIBRA).

Diet preparation

Three diet formulations were done as follows: DRB-C – a control diet formulated with untreated defatted rice bran supplemented with dicalcium phosphate; DRB-PS – a diet formulated with defatted rice bran previously treated with *S. cerevisiae* phytase, and DRB-PN – a diet formulated with defatted rice bran previously treated with Natuphos®. Dicalcium phosphate was added to DRB-C for the purpose of standardizing the content of phosphorus available in the three formulations. The composition of the three diets is shown in Table I. After milling, the feedstuff was mixed and the feeds were pelletized and dried at 50 °C for 24 hours.

Biological assay: animal management and analytical procedure

The biological experiment was conducted at the Departamento de Zootecnia, Universidade Federal de Santa Maria - UFSM, after being approved by the Animal Experimentation Ethics Committee of the UFSM, Case No. 23081.003109/2010-21. Juvenile grass carps (average weight: 57.55 ± 0.4 g) were distributed into nine 280-L tanks (with individual supply, drainage and aeration systems) that were connected to a water recirculating system containing two biological filters and a main reservoir with two thermostats and two resistors for the maintenance of the water temperature. In the 2 weeks prior to the experiment, the fish were fed a commercial feed with 28% of crude protein.

Each treatment was conducted in three replicates and a tank containing 20 fish. The fish were fed at a rate of 3% live body weight divided into three daily feeds, at 8:30, 14:30 and 17:00 h. The feed quantity was adjusted after 15 days by weighing the fish. During this experiment, grass carp was not fed with forage in order to verify

Table I. Composition of the experimental feeds (g kg^{-1} diets)[a].

Feedstuffs	DRB-C	DRB-PS	DRB-PN
Soy meal	430	405	405
Fish meal	50	50	50
Corn	165.4	208.4	208.4
Wheat bran	70	70	70
DRB-C	200	0	0
DRB-PS	0	200	0
DRB-PN	0	0	200
Soy oil	39	39	39
Dicalcium phosphate	18	0	0
Vitamin and mineral premix[b]	15	15	15
Choline	7.5	7.5	7.5
Salt	5	5	5
BHT	0.1	0.1	0.1
Lysine	0.78	1.4	1.4
Methionine + Cysteine	3.02	3.26	3.27
Proximate composition (g kg-^{1})			
Dry matter	926	921	909
Ash	97	78.8	77.8
Crude protein	285	281	277.4
Ether extract	75.4	75.9	74.9
Fiber	195.2	205.5	202.8
Nitrogen free extract	344	358.8	367.1
Calcium (Ca)	9.6	5.2	5.2
Total phosphorus (P)	14.3	11.1	11.0
Available phosphorus	8.3	8.6	8.6
Digestible energy (MJ kg^{-1})	12.09	12.16	12.14

[a]Calculation based on the analysis of the feedstuff.
[b]Vitamin and mineral premix (mg per kg of diet): 18 folic acid; 360 nicotinic acid; 180 panthotenic acid; 0.72 biotin; 72 thiamin; 72 riboflavin; 72 pyridoxine; 5.4 vitamin A; 72 vitamin B12; 720 vitamin C; 0.9 vitamin D3; 180 vitamin E; 36 vitamin K3; 0.15 cobalt; 45 cooper; 750 iron; 1.5 iodine; 300 manganese; 1.5 selenium; 45 zinc.

the effects arising solely from the different formulations of the diet in question. The biological experiment was conducted between February and March with a total duration of 35 days. Fish were weighed at the beginning and the end of the experiment to verify weight gains. Prior to collecting blood samples and removing the organs, the fish were in fasted for 24 hours.

For the blood tests, 27 fish fed on DRB-C, DRB-PS, or DRB-PN diets were anesthetized by immersion in a tri-phenoxyethanol solution (0.03%, diluted in water) the collecting area

massaged to prevent reflux and/or bruising. The blood collection was performed with disposable syringes containing heparin or no anticoagulant, according to posterior analytical requirements. The obtained plasma was used to quantify the levels of total proteins, glucose, triglycerides, cholesterol, calcium, phosphate and alkaline phosphatase through Doles® colorimetric kits (Goiania, GO) and iron serum levels were determined (Doles® colorimetric Kits, Goiania, GO). Plasma magnesium analysis of the fish was also via commercial kit (Katal® Trade Kit).

For the organ analysis, 9 fish fed with DRB-C, DRB-PS, or DRB-PN diets were slaughtered by immersion (ten minutes or more) in tanks containing benzocaine hydrochloride (110 g L^{-1}) before removing the livers and digestive tracts. A homogenization buffer for the digestive enzymes (pH 7.0) was used to prepare an extract of the digestive tract and for the subsequent trypsin and chymotrypsin determination using the methods described by Hummel (1959). The liver analysis provided the glucose (Dubois et al. 1956), the glycogen (Bidinotto et al. 1997) and the total protein determinations by the Bradford method (Bradford 1976).

The chemical composition of whole-fish body was performed with 9 fish collected from each treatment, that were slaughtered, as described earlier, and ground in a food processor for later analysis of the dry matter, ash and protein content, according to the methods described in AOAC (1995), and for fat determination using the method proposed by Bligh & Dyer (1959).

After 20 and 30 days of the experiment, total phosphorus in the tank water was determined colorimetrically by reduction with ascorbic acid, according to the methodology prescribed by the ABNT (NBR 12772/1992). The physicochemical parameters of the tanks water were measured daily (temperature and dissolved oxygen using the YSI digital oxymeter model 550A, Ohio, USA) or weekly (pH with the Digimed pH meter NTA210® (São Paulo, Brazil) and hardness according to Adad (1982). A water sample was collected from biological filters for analysis. The results for the physicochemical water parameters were: a temperature of 26.0 ± 1.8 °C, dissolved oxygen of 5.6 ± 0.5 mg L^{-1}, a pH of 6.9 ± 0.1 and hardness of 20 ± 0.8 mg L^{-1} $CaCO_3$.

The highest content of phosphate, as expected, was found in the waters of the control formulation tank (DRB-C = 1.38 ± 0.14 mg L^{-1}) and was due to the dietary supplementation of inorganic phosphate resulting in concentration higher significantly than DRB-PS and DRB-PN tanks (1.09 ± 0.18 and 1.21 ± 0.14 mg L^{-1}, respectively). The phosphate levels in the water of tanks at 20 days of experiment of all treatments were 1.13 ± 0.20 mg L^{-1} and at 30 days of experiment were 1.32 ± 0.13 mg L^{-1}, are in accordance with those tolerated by the fish (Baldisserotto 2004).

Statistical analysis

All data were analyzed and subjected to one way ANOVA using the Duncan test ($p < 0.05$) for comparison of means. The Kruskal-Wallis variance analysis was used to evaluate data with a non-parametric distribution. The analyses were performed using SAS version 9.1.

RESULTS

The amino acids composition of the tested diets was approximately the same (Table II). The obtained results showed increased availability of phosphorus after the treatment with phytases, it was reflected in the release of 95% of the phosphorus in both treatments. The total phosphorus content was not significantly different among the three diets ($p > 0.05$) and its value determined in the defatted rice bran

was 2.83 ± 0.09%. The available phosphorus in rice bran treated with *S. cerevisiae* phytase (2.73 ± 0.05%) or treated with Natuphos® (2.72 ± 0.17%) were significantly higher ($p < 0.05$) than rice bran not treated (0.94 ± 0.03%).

Table III shows the biochemical results after 35 days of feeding. We observed a trend to inverse proportionality between the glucose and glycogen determinations in liver and the total protein values in the tissue, the latter being signicantly lower in the fish fed on DRB-C diets ($p < 0.05$). In the fish fed on DRB-PS and the DRB-PN diets, higher concentrations of liver protein were found. Analyses of phosphorus and calcium in the plasma of the fish fed on different diets did not show significant differences ($p > 0.05$) among them. Higher levels for the calcium-phosphorus ration in the DRB-C-fed fish were observed compared to the ratio in those fed on the DRB-PS diet ($p < 0.05$). Furthermore, magnesium, iron, alkaline phosphatase, glucose, protein, cholesterol and triglycerides in fish serum or plasma did not show significant differences ($p > 0.05$) among them. Examination of the trypsin and chymotrypsin activity shows that the diet compositions did not cause significant effects on proteases activity.

At the end of the experiment, differences among the weight gains, among the fish fed on different diets were not significant. Fish fed on the DRB-C diet presented a weight gain of 3.9 ± 0.94 g, while those fed on the DRB-PS and DRB-PN diets were 3.8 ± 1.4 g and 4.0 ± 1.7 g, respectively.

For the fish carcass composition, the contents of moisture, crude protein, and total ash were not statistically different (Table IV, $p > 0.05$). However, the highest content of total lipids was found in the whole-body of fish fed on the DRB-PN diet ($p < 0.05$).

Table II. Composition of amino acids (g kg^{-1}) of the experimental feeds.

Amino acids	DRB-C	DRB-PS	DRB-PN	Grass carp requirement
Lysine	15.4	15.5	15.5	15.0
Methionine+Cystine	10.7	10.8	10.8	9.0
Threonine	9.5	9.2	9.2	10.1
Tryptophan	3.6	3.5	3.5	2.4
Valine	12.8	12.4	12.4	13.6
Isoleucine	11.1	10.7	10.7	11.8
Leucine	19.9	19.5	19.5	19.8
Phenylalanine	12.5	12.1	12.1	10.9
Histidine	6.3	6.1	6.1	6.7
Arginine	19.1	18.4	18.4	16.8

Table III. Biochemical parameters of juvenile grass carp (*Ctenopharyngodon idella*) fed on diets with different phytase sources.

Analyses[1]	DRB-C	DRB-PS	DRB-PN
Blood analyses (n = 27)			
Calcium (mg dL^{-1})	6.09 ± 0.48	5.55±0.41	5.77±0.17
Phosphorus (mg dL^{-1})	3.55 ± 0.36	4.37±0.38	3.72±0.38
Ca/P ratio	1.90 ± 0.12[a]	1.41±0.08[b]	1.65±0.14[ab]
Magnesium (mg dL^{-1})	2.32 ± 0.16	2.65±0.19	2.57±0.12
Iron (µg dL^{-1})	31.52 ± 2.01	34.25±2,81	31.88±2.04
Phosphatase (UI L^{-1})[2]	29.06±2.97	32.05±2.19	33.38±1.73
Glucose (mg dL^{-1})	72.19±8.17	82.89±7.57	76.27±7.33
Protein (g dL^{-1})	1.95±0.13	1.85±0.20	1.70±0.18
Cholesterol (mg dL^{-1})	170.23±16.01	135.93±10.54	169.63±16.84
Triglycerides (mg dL^{-1})[3]	187.14±18.62	179.69±21.33	170.27±14.78
Hepatic analyses (n = 9)			
Glucose (µmol g^{-1} of tissue)	432.02±36.95	295.08±64.74	318.56±41.56
Glycogen (µmol g^{-1} of tissue)	1267.14±202.33 [a]	677.14±37.76 [b]	679.65±35.34[b]
Protein (mg g^{-1} of tissue)[3,4]	22.11±2.14[b]	52.51±4.04[a]	57.01±1.15[a]
Intestinal analyses (n = 9)			
Trypsin (UI mg^{-1})[5]	7.62±0.59	7.95±0.47	6.77±0.34
Chymotrypsin (UI mg^{-1})[5]	3069.16±280.19	3387.07±199.45	2884.75±144.00

Different letters within the same line indicate significant differences between the treatments (Duncan test p<0.05).
[1] Results presented as mean ± standard deviation.
[2] One UI of phosphatase is the enzyme amount that catalyzes the hydrolysis of 1µmol of substrate/minute/liter of serum.
[3] Variance analysis of Kruskall-Wallis (nonparametric).
[4] Variance significance level: (p<0.0001).
[5] One UI of enzyme is the amount of enzyme that catalyzes the hydrolysis of 1µg of substrate/minute/mg of protein.

Table IV. Proximate chemical composition of whole body (% on fresh weight basis) of juvenile grass carp (*Ctnopharyngodon idella*) fed on diets with different phytase sources.

	DRB-C	DRB-PS	DRB-PN
Analyses[1]			
Dry matter	25.68±0.70	25.19±0.48	26.42±0.51
Moisture	74.32	74.81	73.58
Crude protein	12.64±0.18	12.95±0.16	12.90±0.17
Total lipids	9.23±0.41[b]	9.37±0.44[b]	10.56±0.33[a]
Total ash	2.47±0.10	2.43±0.11	2.54±0.11

[1]Results presented as mean±standard deviation (n=9).
Different letters within the same line indicate significant differences between the treatments (Duncan test p <0.05).

DISCUSSION

The phytases catalyze phytic acid hydrolysis and the hydrolyzed inorganic phosphate is used as a measure of the available phosphorus in the rice bran for fish nutrition. The data from the effective action of phytase at a concentration of 550 FTU kg^{-1} of DRB were higher than those obtained in similar studies (Jackson et al. 1996, Furuya et al. 2001, Liu et al. 2014). However, those studies did not evaluate the bioavailability of the phosphorus. Values for the total phosphorus content determination reported in the literature vary: 1.5% (NRC 1994) or 2.33% (Moreira et al. 2003).

Cúneo et al. (2000) evaluated the dephytinization of soybean meal with phytase and observed a reduction of only 37% of the phytic acid content. The experiment using treated soy concentrate as a basis for fish diets conducted by Storebakken et al. (1998) resulted in 64% and 70% increases in phosphorus solubilities after 10 and 60 minutes of incubation, respectively. Schons et al. (2011) reported that the treated sorghum with phytase and tannase was better than raw sorghum in the apparent digestibility of phosphorus and in biochemical indices for rats.

Phosphorus availability in diets containing soybean meal supplemented with commercial phytase (Natuphos® BASF 5000 FTU g^{-1}) used to feed Nile tilapia, *Oreochromis niloticus* was 65.23% (500 FTU kg^{-1} of diet) and 72.63% (1500 FTU kg^{-1} of diet), whereas in the treatment without the enzyme, the availability of the mineral was 38.21% (Furuya et al. 2001). In a study conducted to evaluate the effect of Natuphos® phytase on phosphorus bioavailability of rice bran in diets for broiler chickens, bioavailability levels were 51.54% and 61.31% using 400 and 800 FTU kg^{-1}, respectively (Conte et al. 2002).

It is important to mention the addition of dicalcium phosphate to the control formulation (DRB-C) for the purpose of standardizing the content of phosphorus available in the three formulations. The diets presented phosphorus contents above the requirements established by the National Research Council (NRC 1993) for *Cyprinus carpio* (0.6%) and a similar final composition in regard to other nutrients.

The metabolic responses observed for the DRB-PS diet are similar to those obtained with the use of the commercial enzyme, which is widely applied in fish feeding. There were no significant differences in content of magnesium, iron, alkaline phosphatase, glucose, protein, cholesterol, or triglyceride in the blood and that suggests that the pretreatment of DRB with phytases did not interfere in the normal concentrations of those variables.

The results of phosphorus and calcium, which did not differ among the three evaluated treatments, diverge from a similar study conducted by Moreira et al. (2003), in which the highest phosphorus concentrations in the plasma were detected in the control treatment without phytase and supplemented with inorganic phosphate. The presence of phosphorus in the diets is important for the fish growth and metabolism. An increase in growth and prevention of carp skull deformities were observed when mineral was added to the diets (Halver & Hardy 2002). Phosphorus is intensely involved in metabolic processes, participating in all reactions in which energy consumption is needed. Thereby, when the intake of phosphorus deficient feed occurs, the physiological system promotes the recycling of bone phosphorus, reduces excretion and increases absorption, affecting the animal performance at high levels of deficiency (Moreira et al. 2003).

Considering the levels determined for calcium, it is noteworthy that its supply in fish diets is of secondary importance due to the most of the calcium used in fish metabolism is obtained from the captured water via the

gills. Calcium has important functions related to muscle contraction, blood clotting, the transmission of nerve impulses, the maintenance of cell membrane integrity controlling membrane permeability, and the nutrient uptake of cells (Halver & Hardy 2002).

The higher levels for the calcium-phosphorus ratio in the DRB-C-fed fish may be due to the dicalcium phosphate supplementation of this formulation. Special concern is needed with the calcium content when lowering the phosphorus concentrations of diet. Calcium presents a lower affinity to bind with phytate; however, this mineral is found at higher levels in the diets. A total Ca/P ratio of 2:1 or higher reduces phytate utilization and the absorption and performance of these minerals. Phosphorus metabolism is directly related to calcium metabolism but its variations in the plasma are small due to efficient physiological mechanisms that involve the parathyroid hormone, calcitonin and vitamin D (Halver & Hardy 2002, Moreira et al. 2003).

The higher concentrations of liver protein on DRB-PS and the DRB-PN diets may have been due to better protein utilization and better amino acid availability caused by phytase action during the pre-treatment of rice bran. It is well known that the presence of phytic acid in diets affects protein digestibility due to the formation of insoluble complexes. Thus, the hydrolysis of complex nutrients by phytase could contribute to obtain a diet with superior nutritional quality and increased nutrient digestibility. The pre-treatment with phytase of soy protein concentrate in diets for Atlantic salmon (*Salmo salar*) resulted in a reduction of phytic acid and in higher digestibility rates and protein retention (Storebakken et al. 1998).

Liver glycogen and glucose levels were lower in the fish in DRB-PS and DRB-PN regimes probably due to their preferential use to meet the energy demands of animal while preserving its protein stocks. The lipid levels of the body composition were lower than those determined for Atlantic salmon (*Salmo salar*) (Sajjadi & Carter 2004). Those authors reported that fish fed with phytase-added diets, compared to those fed with formulations containing phytic acid, presented higher levels of lipids.

Phytase levels comparable to those applied in this study provided satisfactory results in similar studies. The inclusion of 500 FTU kg^{-1} of feed was sufficient to ensure an adequate performance and the deposition of phosphorus in the bones for channel catfish (*Ictalurus punctatus*) as reported by Jackson et al. (1996). Furuya et al. (2001) observed that the best results in terms of performance, bone mineral retention, and digestibility were obtained with a phytase supplementation of 700 FTU kg^{-1} of feed in diets for Nile tilapia. Moreira et al. (2003) reported that the use of 759 enzyme units in diets based on corn, soybean meal and defatted rice bran for pigs, during their growth phase, makes it possible to eliminate traditional phosphorus sources. In their study, the DRB had an inclusion level of 20% in the diets and showed the advantage of pretreating the bran with phytase (500 FTU kg^{-1}) over supplementing it with inorganic phosphate.

The present study showed that trypsin and chymotrypsin activities are insignificantly affected by phytase source. Previously to the present study, Sajjadi & Carter (2004) found no effect on trypsin activity after including phytic acid in the diets of Atlantic salmon (*Salmo salar*). Thus, it could be suggested that there was poor digestibility and protein deposition associated to that formulation. The absence of any effects on trypsin and chymotrypsin activity was expected in this experiment since the formulations had similar nutrient composition and the same concentration. Any increase of digestive enzymes in the grass carp that occurs is attributed to increased feed availability, as they require those

enzymes for digestion and absorption (Das & Tripathi 1991). Lazzari et al. (2007) also emphasize that the alkaline proteases, in particular, do not respond linearly to the dietary protein level, being more susceptible to the presence of inhibitory substances and being reliable indicators of the nutritional status of fish when associated to metabolic parameters. A significant reduction of trypsin activity has only been observed in the larvae of the common carp (*Cyprinus carpio*) fed with diets containing high levels of soy protein concentrate and soybean trypsin inhibitors (Escaffre et al. 1997). The determined trypsin and chymotrypsin values of this experiment were higher than those found for silver catfish (*Rhamdia quelen*) fed with a similar diet content of crude protein (27%) (Lazzari et al. 2007).

It is noticed that no changes were observed in contents of moisture, crude protein, and total ash due to phytase supplementation except total lipids, which was higher in the whole-body of fish fed on the DRB-PN diet ($p < 0.05$). These results are much more expected because of the similarity of diets formulation and composition. It is known that changes in protein and lipid contents in fish body could be linked to changes in their synthesis and/or deposition rate in muscles (Fauconneau 1984, Abdel-Tawwab et al. 2006).

The cumulative effect of phosphorus excretion into the water tanks may also be observed in the present study. The phosphorus level, after 30 days of experiment, was higher ($p < 0.05$) than the value determined after 20 days, showing that continuous excretion occurred into the aquatic environment. The implications of phytase use on the lower nutrient discharge in fish farming tanks have already been mentioned by other authors and, despite being an essential element in fish feeding, the phosphorus concentration of the diet should meet the necessary performance requirements without impair the quality of the water used for cultivation (Gonçalves et al. 2007).

CONCLUSIONS

The effect of enzyme treatment on the rice bran used in feed preparation for grass carp (*C. idella*) were striking. It could be suggested that the use of phytase at a concentration of 550 FTU kg^{-1} of rice bran could replace supplementation with external phosphorus sources and may also improve nutritional quality, thereby benefiting the fish performance. Phosphorus availability of the DRB resulting from the phytase inclusion in diets irrespective to its source also contributes to the reduction of environmental pollution as it significantly reduces the excretion of unused phosphorus.

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