COMMERCIAL PHYTASE USE IN PACU (*Piaractus mesopotamicus*)

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ABSTRACT

Phosphorous is one of the most essential nutrients for fish. However, an excess of this mineral in fish diets in phytate form can lead to higher levels of excreted phosphorous. The phytase enzyme is responsible for the conversion of phytate into organic phosphorous, although it has been suggested that fish do not produce this compound, requiring the use of a commercial enzyme . In this context, Natuphos® effects on pacu were evaluated through plasma osmolality and ionic level assessments to verify if commercial phytase is able to reduce phosphorous excretion by chelate cleavage, for example. **Results:** The best composition to be applied as fish feed additive for Pacu is a plant protein source containing 0.39% total phosphorus supplemented with 3000 phytase units/kg in liquid form. **Conclusion:** Phytase promotes increases in magnesium concentrations only, suggesting chelate cleavage improves phosphorus absorption. The application of phytase in other species should be considered.

Keywords: fish meal, meal composition, ions, phosphorous

USO DE FITASE COMERCIAL EM PACU (*Piaractus mesopotamicus*)

RESUMO

O fósforo é um dos principais nutrientes para o organismo do peixe. Porém, uma alta concentração deste em rações, usado na forma de fitato, pode resultar em uma alta excreção de fósforo no ambiente. A enzima fitase é responsável por converter o fitato em fósforo orgânico, mas sugere-se que ela não exista naturalmente em peixes. Assim, fitase comercial pode ser utilizada. Neste contexto, os efeitos da Natuphos® em Pacu foram avaliados em relação à osmolalidade do plasma e concentrações iônicas para avaliar se a fitase comercial diminui a excreção de fósforo, pela quebra de quelatos, por exemplo. **Resultados:** Para o Pacus, a melhor composição a ser usada como aditivo em rações é a derivada de vegetais com 0.39% de fósforo totais e suplementada com 3000 unidades de fitase/kg na forma líquida. **Conclusão:** O uso de fitase spromoveu apenas o aumento na concentrações de magnésio, sugerindo a quebra de quelatos para melhorar a absorção do fósforo. É importante considerar os resultados do uso de fitase em outras espécies.

Palavras–chave: ração de peixes, composição de rações, íons, fósforo

INTRODUCTION

Constant fish farming diversification is currently employed to maintain high fish production levels, including environmentally friendly resources and tools such as fish growth promoters (Zheng et al. 2019). However, costs, mainly concerning feed, have increased significantly (Kim et al., 2019). In pursuance of cost reductions and the need for adequate fish nutrition (Pezzato et al., 2009), fish meal manufacturers

have sought out protein concentrates from plant-derived based meals (Weiler et al., 2019; Santos et al., 2020), which contain high amounts of phytate as a phosphorous source. Phytate can comprise almost 80% of the total phosphorous in plants (Santos et al., 2020). One of the most relevant native species employed Brazilian fish farming is the Amazonian freshwater species *Piaractus mesopotamicus*, commonly named Pacu (Signor, 2011). Fish farming success depends heavily on the physiological knowledge of the farmed species (Evans, 2005; Baldisserotto, 2009), such as the way fish utilize meal nutrients.

In freshwater environments, phosphorous is retained by fish by transporters such as Na+/Pi exchangers, or simply by diffusion (Baldisserotto, 2009). This element is essential for many crucial functions in vertebrates, such as bone mineralization, nucleic acid building and synthesis, processes involving cellular chemical energy (Souza, 2004), as well as a coenzyme and phospholipid composition factor and in the fat, carbohydrate, and amino acid metabolisms (Jobling, 2012). This compound is also critical for growth and reproduction (Roy & Lall, 2003). A phosphorous-poor diet can reduce the levels of other minerals in fish, such as magnesium (Deschamps, 2016), which can induce skeletal deformities (Lall & Lewis, 2007). In addition, poor phosphorous digestion and absorption may also increase phosphorous excretion (Jacobsen & Borresen, 1998).

It is common to add phosphate and other ions, such as Na+ (e.g.: monosodium phosphate) to fish meal. This mixture usually improves fish meat texture, as these nutrients promote tissue hydration (Sampaio et al., 2001). In general, freshwater fish require from 0.4 to 1.57% /kg dietary available phosphorous (Kubtiza, 1999; Kumar, 2012). Furthermore, fishmeal diets, generally applied to tropical fish, result in higher phosphorus retention levels in phytate form (Lao, 1991; Cao et al., 2007; Deak & Johnson, 2007; Zheng et al., 2019). Phytate is a salt formed by phytic acid, a non-digestible ester for animals (Kubtiza, 1999) that can form insoluble complexes, which, in turn, decrease the action of proteolytic enzymes, an undesired fact for fish, due to their anti-nutritional properties (Maas et al., 2018). This compound may also reduce protein, fat, and mineral digestibility (Nwanna et al., 2008), and undigested phytate also contributes to environmental eutrophication (Baruah et al., 2007).

Phytase is an enzyme that cleaves phytate, and is not produced by fish (Greiling et al., 2019). The use of this enzyme increases the availability of phytate and of several minerals (Roy et al., 2014; Morales et al., 2014; Lemos & Tacon, 2017). Further benefits for fish, beyond worldwide pressure to reduce discharged phosphorous, have led to the increased use of this enzyme (Norag et al., 2018). In this regard, phytase increases the phosphorus metabolism, as it converts phytate into organic phosphorus (Cao, 2007) and breaks down phosphorous-chelated nutrients , increasing phosphorous retention (Cao et al., 2007; Kumar et al., 2012; Shah et al., 2016). Therefore, this enzyme enables phytate access, resulting in decreased phosphate excretion and, consequently, decreasing environmental phosphorous pollution (Bedford, 2000). Because of this, exogenous enzymes such as phytase, are routinely employed in fish farming to provide higher fish meal quality, i.e.,, providing proteins required for different fish growth stages (mainly during larval development), and for immune performance (Zheng et al., 2019). According to Drew et al. (2005), endogenous enzymes can be supplemented by the appropriate addition of exogenous enzymes. The question this paper raises in this regard it, what is the best phytase addition composition to different plant-derived fish meals?

MATERIAL AND METHODS

This experiment was approved by CEUA: n° 37/2014. Pacu specimens (*Piaractus mesopotamicus*) (5.11 ± 0.26 cm, initial weight 1.93 ± 0.25 g and final weight 38.3 ± 3.48 g) were purchased from a fish farm in Palotina, Paraná, Brazil (Sgarbi Tropical Fish Farms, latitude 24°18'S, longitude 53°48'W). Fish acclimation and assays were performed at the Fish Nutrition Laboratory belonging to the Federal University of Paraná (UFPR). The fish were maintained for 75 days in separate aquaria identified according to the applied meal treatment after acclimatization. Thirty polyethylene boxes (300 L, comprising 15 fish/box) were mounted as a recirculating system under both mechanical and biological filtration, constant aeration, and a natural photoperiod. Fish were fed *ad libitum* twice a day. Each meal was distributed in six treatments comprising five repetitions each. The basal experimental diet comprising soybean meal and corn employed herein was formulated as an isoenergetic and isoproteic feed containing 3200 kcal.kg-1 of digestible energy and 26% of crude protein, respectively. Ingredients were homogenized using an experimental mixer (G.PANIZ® model BP 12C - Brazil) with an 8 kg.h-1 capacity. The mixture was then processed employing an experimental extruder (EXTEEC® - Brazil) with a 15 kg.h-1 production rate, using a 3.0 mm matrix. Granulated phytase was first added to the mix, while liquid phytase was added after pellet extrusion and drying, diluted in 4 mL of water per kg of meal, and added through aspersion using a hand pump.

The fish meal compositions were based on experiments reported by França (2015). The negative control (A) did not contain monosodium phosphate and phytase, and presented 0.42 % total phosphorous (TP). The positive control (B) did not contain phytase, but contained inorganic phosphorus (monosodium phosphate, $NaHPO_4$) at 2%, with 0.75% TP. Natuphos® (Company Basf), a commercial additive used in plant-derived meals, comprised 5000 FTU/g in granulated form and 10000 FTU/L in liquid form. Phytase activity is expressed as Phytase Units (FTU), comprising the amount of phytase required to release 1 µmol of inorganic phosphate per minute (Greiling et al., 2019). Meals in this treatment were composed of 0.43% TP and 1500 FTU/kg in granulated form (C); 0.39% TP and 3000 FTU/kg in granulated form (D); 0.41% TP and 1500 FTU/kg in liquid form (E), and 0.39% TP and 3000 FTU/kg in liquid form (F). Following the experimental period, blood samples were collected by caudal vein puncture (three fish per treatment, comprising 15 fish in total), and plasma was obtained by centrifugation (3,000 RPM/5 min). Samples were then sent to the Comparative Osmoregulation Physiology Laboratory (LFCO) in Curitiba, where they were maintained at -20 ºC until the osmolality and ionic assays were performed.

Plasma osmolality was determined in undiluted samples through a vapor pressure osmometer (VAPRO 5520, Wescor, USA). Plasma phosphorous, chloride, and magnesium ions were assayed through colorimetric methods using commercial kits (Labtest, Lagoa Santa, Brazil) employing an Ultrospec 2100 PRO spectrophotometer (Amersham Pharmacia Biotech, Sweden), where absorbances were determined at 650, 470 and 505 nm, respectively. Sodium and potassium ionic levels were determined using flame photometry (CELM FC-180, Brazil) in samples appropriately diluted in ultra-pure water (1:200). All statistical analyses were performed using the Sigma Plot v. 11.0 software. One-way ANOVAs (fish meal) (α= 5%) were applied to the assayed parameters, followed by the Holm-Sidak or Tukeypost hoc tests, as suggested by the statistical software.

RESULTS

Plasma osmolality (~310 mOsm/kg H_2O) (Figure 1a), Na^+ levels (~149 mM), Pi levels (~9.0 mM), and K^+ levels (~3.0 mM) were unaffected by the different fish meals (Figure 1b and Figure 2), while Cl^- levels (~140 mM among all groups) were lower in fish fed the "C" meal (130 ± 3.97 mM). The Mg^{+2} levels (~1.1 mM among all groups) were higher in fish fed the "F" meal (1.26 ± 0.06 mM) compared to those fed the "A" meal: without phytase or phosphorous 152 4.56 mM (Figure 1b) (0.99 ± 0.04 mM) (Figure 2).

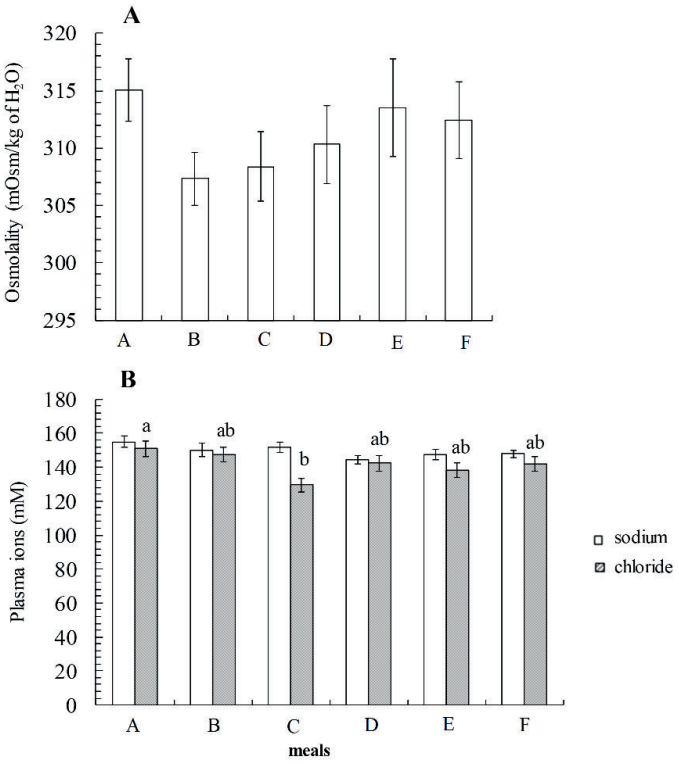

Figura 1. Osmolality (A) and Plasma ions – sodium and chloride (B) in Pacu after different feeding. The letters A to F means the kind of meal (see in Material and Methods above). Different letters at columns shows differences at chloride levels among groups.

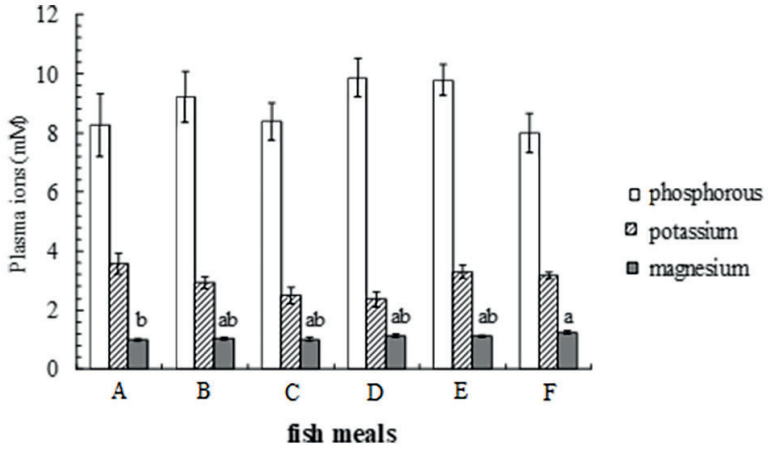

Figura 2. Plasma ions in Pacu after different feeding. The letters A to F means the kind of meal (see in Material and Methods above). Different letters at columns shows differences at magnesium levels among groups.

DISCUSSION

Total osmolality, plasma sodium, phosphorous, and potassium were equally well regulated in all investigated groups (Evans, 2005), even in the "C" meal treatment, which exhibited lower Cl- levels. However, one treatment, namely the "F" meal treatment, affected Mg2+ levels, which were higher in fish fed 0.39% TP and 3,000 FTU/kg in liquid form when compared to the negative control ("A" meal treatment). Is important to note that this meal also contained lower phosphorous levels compared to other meals, except for the "D" meal, comprising the same composition but in granulated form. Phytase can optimize mineral retention through the hydrolysis of mineral chelates (such as magnesium) with phosphorus, increasing the availability of this nutrient (Cao, 2007). The literature, however, does not report phytase effects on Mg2+ and Pi plasma levels.

Two studies on phytase experiments in Pacu were considered herein for discussion. In the first, P. mesopotamicus were fed a plant-derived meal containing 0.40% TP, meeting

the food requirements for the species (Signor et al., 2011). Mineral retentions of iron, zinc, and phosphorous were higher in fish fed 8 g/kg phytic acid and phytase (optimal condition 3935 FTU/kg), which also displayed greater iron bioaccessibility (Cian et al., 2018). It is important to note that offered phytase levels were higher than those offered herein . The other study on Pacu offered phytase three times a day for 60 days (Natuphos® 30% protein, 0.31 % TP and 500, 1000, or 2000 FTU/kg phytase), with optimal supplementation of ~433 FTU/kg phytase (Furuya, 2008). TP levels, however, in that study were lower than in the present assessments, which employed normal phosphorous levels in fish meal.

Some controversial results have been reported regarding phytase supplementation for other fish species, discussed herein with regard to zootechnical performance parameters (Table 1). For example, for the catfish Rhamdia quelen, another native Brazilian species, 1500 FTU/kg of a phytase-like additive did not result in any positive fish body performance effects (growth and feed efficiency), hematological parameters, or bone composition (Weiler et al., 2019). However, according to Rodrigues et al. (2020), other studies have reported better body performance following phytase addition. For Pangasius pangasius fingerlings, for instance, supplementation with 500 FTU/kg or higher can improve fish weight gain, growth, protein efficiency, apparent protein utilization, and energy retention (Debnath et al., 2005).

Another study was conducted on Nile tilapia Oreochromis niloticus, where fish were fed a diet containing 50% (0.48% TP) of the total required phosphorus supplemented with 500 or 1000 FTU/kg, resulting in increased body weight gain and nutrient utilization (Norag et al., 2018). Another study on the same species employed 500 FTU/kg phytase and 28% of proteins and reported weight gain, protein efficiency, and mineral retention, as well as decreased P excretion (Furuya et al., 2005). In yet another study on Nile tilapia, fish were fed 1000 FTU/kg phytase for 38 days, which improved growth, dry matter digestibility, crude protein, carbohydrates, energy, phosphorus, and calcium, also indicating a synergism with xylanase, which cleaves xylose (Maas et al., 2018).

Tabela 1. Search of papers founds with key words fish (es)/ Pacu + phosphorous, fish (es)/ Pacu + phytase.

Species	Habitat/ experimental water	Family/Order	P in diet (plant derived)/ feed time	P excreted
Piaractus mesopotamicus	FW / FW	Characidae/ Characiformes	0,39% TP*, 75 days: 2/day	not analyzed
			x, 38 days: 2/day	not analyzed
			0,31% TP, 60 days: 3/day	not analyzed
Rhamdia quelen	FW/FW	Heptapteridae/ Siluriformes	x, 90 days: 4/day	not analyzed
			x, 90 days	not analyzed
Pangasius pangasius	FW/FW	Pangasiidae/ Siluriformes	x, 30 days	not analyzed
Oreochromis niloticus	FW, BW/ FW	Cichlidae/ Perciformes	x, 38 days	increase P digestibility
			0,48% TP, 60 days: 2/ day	not analyzed
			x, 60 days	7.17 kg ³/t fish (16.15 without phytase)
Cromileptes altivelis	SW/ SW	Serranidae/ Perciformes	x, 90 days: 2/day	not analyzed
Lateolabrax japonicus	CAT***/SW	Lateolabracidae/ Perciformes	0,81% TP, 60 days: 2/day	4.1 (6.8without phytase) g P kg^{-1}
Pagrus major	SW/SW	Sparidae/ Perciformes	~13 g/kg diet, 80 days: 2/day	increase P digestibility
Sparus aurata	SW, BW/ ?	Sparidae/ Perciformes	1.45% TP,90 days: 2/day	0.6% in feces
Cyprinus carpio	FW, BW/ FW	Cyprinidae/ Cypriniformes	x, 72 days: 3/day (3% of total weight)	not analyzed
			x, 42 days: 3/ day	increase P digestibility
			x, 60 days: 4/day	not analyzed
Labeo rohita	FW, BW/ FW	Cyprinidae/ Cypriniformes	14.05 mg/kg, 60 days: 2/day	PHY: increase P digestibility
			1.98%, 60 days (3% of total weight)	P digestibility: ~35 to ~64 (1.5% CA) and ~77% (3% CA)
			0.6 TP, 60 days: 2/day (3% of total weight)	increase P digestibility
Oncorhynchus mykiss	ANA**/FW	Salmonidae/ Salmoniformes	low P diet, 72 - 83 days	reduce 19 and 45% P excretion
			7.10 g, 2/ day	increase P digestibility (P digested: 1870 to 4860)
			x,90 days: 2/day	not analyzed
Salmo salar	ANA/SW	Salmonidae/ Salmoniformes	7.10 g, 3/day	increase P digestibility (P digested: 898 to 1908)
			* reccomended meal	**ANA:anadromous, *** CAT: catadromous

Phytase added: LF (liquid), G (granulated)	Obs (effect, etc)	Reference
Natuphos: 3000 FTU/ kg*, L	higher Mg^{+2} levels	*this study*
Novozyme: 3935 FTU/kg*, G	greater iron bioacessibility	Cian *et al.*, 2018
Natuphos: ~ 433 FTU/ kg*, G	weight gain, proteic eficiency, feed convertion	Furuya, 2008
Natuphos: 1500 FTU/ kg, G	positive effect: weight gain, proteic eficiency, feed convertion/ hematological parameters and bone c	Rodrigues *et al.*, 2020
Natuphos: 1500 FTU/ kg, G	no effect: weight gain, proteic eficiency, feed convertion/ hematological parameters and bone comp	Weiler *et al.*, 2019
Natuphos: 500* FTU/ kg,G	positive effect: weight gain, growth, protein efficiency, apparent protein utilization and energy reten	Debnath *et al.*, 2005
1000 FTU/ kg, G + xylanase 4000 U/kg	positive effect: growth, digestibility of dry matter, crude protein, carbohydrates, energy, phosphorus	Maas *et al.*, 2018
Quantum Blue: 500 and 1000 FTU/ kg, G	increased body weight gain and nutrient utilization	Norag *et al.*, 2018
Natuphos: 500* FTU/ kg, G	positive effect: weight gain, protein efficiency and mineral retention	Furuya *et al.*, 2005
490 - 602* FTU/ kg, G	growth and food conversion ratio	Samidjan and Rachmawati, 2017
DSM: 500 FTU/ kg G + 400 mg cellulase + 800 mg xylanase	positive effect: growth, P retention	Ai *et al.*, 2007
Natuphos: 2000* FTU/ kg G	positive effects: growth performance, retention ptn efficiency, P digestibility	Biswas *et al.*, 2019
2g/ kg diet G	improve growth performance	Ayhan *et al.*, 2018
Phyzyme XP: 1000 FTU / kg G + 3% od citric acid*	positive effect: weight and growth	Zugarkova *et al.*, 2018
Natuphos: 943 and 1100* FTU/ kg, G	positive effect: increase P and ptn digestibility (1100 FTU), efficiency in feed utilization, increase nutr	Rachmawati and Samidjan, 2017
500 FTU/ kg, G + 30 g/ kg citric acid	positive effect: hematocrit and growth performance, more ash and less lipid in muscle composition	Khajepour *et al.*, 2012
1000 FTU/ kg, G+ 2% citric acid	positive effect: ions digestibilities and growth rate, feed conversion ratio.	Afzal *et al.*, 2019
Phyzyme: 1000 FTU/kg*, G + 1.5 and 3% citric acid	positive effect: growth performance, increase nutrient digestibility	Habib *et al.*, 2018
Natuphos: 500 FTU/ kg, G + 3% citric acid	positive effect: growth performance	Baruah *et al.*, 2007
2500 FTU/ kg*, G and 11 or 15°C	positive effect: weith gain; negative effect: growth and feed eficiency	Lee *et al.*, 2020
Ronozyme: 2800 FTU/ kg, G	inositol-6-phosphate (InsP-6) disappearance: 8.4 to 79.9%	Greiling *et al.*, 2019
2g/ kg diet, G	negative effect: growth and feed conversion ratio, lipid and ptn digestibility	Yigit *et al.*, 2016
Ronozyme: 2800 FTU/ kg, G	inositol-6-phosphate (InsP-6) disappearance: 8.6 to 22%	Greiling *et al.*, 2019

In a study on the humpback grouper Cromileptes altivelis, the best fishmeal composition consisted in the addition of 490 - 605 FTU/ kg, resulting in improved growth and food conversion ratios (Samidjan & Rachmawati, 2017). For Japanese sea bass, Lateolabrax japonicas, 500 FTU/ kg of phytase along with other carbohydrate enzymes, promoted higher growth rated and reduced P excretion (Ai et al., 2007). In red sea bream, Pagrus major, improvements in final fish mean weight, phosphorous digestibility and retention efficiencies for protein, lipid, and phosphorous were noted when fish were fed a diet comprising 80% of a soy protein concentrate with 2000 FTU/kg (Biswas et al., 2019). In seabream, Sparus aurata, a 2 g/kg diet of phytase improved growth and reduced P excretion in fish feces (Ayhan et al., 2018).

For common carp, Cyprinus carpio, one study indicated that optimum phytase doses for promoting growth and nutrient utilization range from 943 to 1100 FTU/kg (Rachmawati & Samidjan, 2017). In another study, 500 FTU/kg and 1000 FTU/kg and citric acid (3%), that optimizes pH in guts for enzyme activity, promoted a 20% lower feed conversion ratio and 11% higher growth rate in the same species compared to the control group (Zugarkova et al., 2018). The addition of citric acid (30 mg/kg) and phytase (500 FTU/kg) also increased growth and reduced the muscle lipid content in this species (Khajepour et al., 2012). In rohu, Labeo rohita, 2% citric acid and 500 FTU/kg of phytase increased P digestibility from ~61 to 83% (Baruah et al., 2007). Also in rohu, calcium, magnesium, copper, zinc, sodium, potassium, iron, and manganese digestibilities were enhanced by the addition of citric acid (2%) and 1000 FTU/kg of phytase, also comprising the same composition that promoted the maximum growth rate (Afzal et al., 2019). The same results were noted for 1.5 and 3% of citric acid and 1000 FTU/kg phytase, in addition to increased phosphorous digestibility (Habib et al., 2018).

Salmonids displayed better (Cain and Garling, 1995) or equal (Cain & Garling, 1995; Sajjadi & Carter, 2003) growth and feed conversions when fed phytase compared to control groups. One study fed Oncorhynchus mykiss and Salmo salar fish meal containing 3.89 g/kg mono ammonium phosphate and 2800 FTU/kg, reporting that phytase increased P digestibility in S. salar by 16% to 30%. while phytase alone increased P digestibility in O. mykiss, ranging from 33% to 74%. The higher digestibility observed in rainbow trout may be due to the species stomach pH, which is lower in freshwater fishes than in seawater fishes (Greiling et al., 2019). Furthermore, phytase (2 g/kg diet) did not affect growth or feed efficiency of O. mykiss (Yigit et al., 2016; Hiscocks et al., 2020), although a low P-diet containing 2,500 FTU/kg phytase reduced phosphorous excretion by 19% (11°C) and 45% (15°C) , with a weight gain of 36 and 45% at those respective temperatures (Hiscocks et al., 2020).

Differential enzyme efficiencies may be affected by rge type of phytase (different isomers) (Greiling et al., 2019), species (probably due to a diversity of digestive systems) fish meal composition, temperature and pH levels, enzyme-substrate specificities, and gastrointestinal tract inhibitors. In addition, different protocols, even grinding meal degree (Li et al., 1996), have also been noted as leading to different results (Zheng et al., 2019)

The positive results regarding phytase addition are due to the phytate complexe cleavage, which also releases other chelated nutrients important for body development (Baruah et al., 2005). In our study, 0.39% TP (total phosphorous) and 3,000 FTU/kg liquid form meal affected magnesium levels, indicating the importance of the aforementioned cleavages. This diet may improve phosphorous digestion. Thus, the use of TP alongside phytase in liquid form, aiming at the best meal absorption, as well as P. mesopotamicus feeding habits may be responsible for these results.

The findings reported herein indicate the need for further studies on the relationship between P and phytase supplemented in fish meals and on different fish homeostasis and zootechnical performance aspects, as well with regard to lesser environmental impacts due to P excretion.

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REFERENCES

AFZAL, M., NIGHAT, S., ALI, H., SYED ZAKIR, S., MAHROZE, F., SYED, H., MUHAMMAD, B. & MAJID, H. (2019). Effectiveness of acidification and phytase pretreatment on growth performance, muscle proximate composition, and nutrient digestibility of rohu (Labeo rohita, Hamilton 1822) juveniles fed soybean meal-based diet. Zool. Soc. Pak.: 51 (5), 1741–1750. Doi: http://dx.doi.org/10.17582/journal.pjz/2019.51.5.1741.1750

AI, Q., MAI, K., ZHANG, W., XU, W., TAN, B., ZHANG, C., & LI, H. (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, Lateo labrax japonicus. Comp. Biochem. Phys. Part A.: 147(2), 502–508. Doi: https://doi.org/10.1016/j.cbpa.2007.01.026

AYHAN, V., DILER, I., ARABACI, M. & SEVGILI, H. (2018). Enzyme supplementation to soybean-based diet in gilthead sea bream (Sparus aurata): Effects on growth parameters and nitrogen and phosphorus excretion. Kafkas Üniv. Vet. Fak. Derg.: 14(2), 161–168. Doi: https://doi.org/10.9775/kvfd.2008.33-A

BALDISSEROTTO, B. (2009). Fisiologia de peixes aplicada à piscicultura. Santa Maria: Editora da Universidade Federal de Santa Maria.

BARUAH, K., PAL, A.K., SAHU, N.P., JAIN, K.K., DEBNATH, D. & MUKHERJEE SC (2007). Dietary microbial phytase and citric acid synergistically enhance nutrient digestibility and growth performance of Labeo rohita (Hamilton) juveniles at the sub-optimal protein level. Aquacul. Res.: 38, 109-120. Doi: https://doi.org/10.1111/j.1365-2109.2006.01624.x

BARUAH, K., PAL, A.K., SAHU, N.P., JAIN, K.K., MUKHERJEE, S.C., & DEBNATH, D. (2005). Dietary protein level, microbial phytase, citric acid and their interactions on bone mineralization of Labeo rohita (Hamilton) juveniles. Aquacul. Res.: 36, 803–812. Doi: https://doi.org/10.1111/j.1365-2109.2005.01290.x

BEDFORD, M.R. (2000). Exogenous enzymes in monogastric nutrition their current value and future benefits. Anim. Feed Sci. Tech.: 86(1-2), 1–13. Doi: https://doi.org/10.1016/S0377-8401(00)00155-3

BISWAS, A., ARAKI, H., SAKATA, T., NAKAMORI, T. & TAKII, K. (2019). Optimum fish meal replacement by soy protein concentrate from soy milk and phytase supplementation in the diet of red seabream, Pagrus major. Aquaculture: 506(15), 51–59. Doi: https://doi.org/10.1016/j.aquaculture.2019.03.023

CAIN, K.D. & GARLING, D.L. (1995). Pre-treatment of soybean meal with phytase for salmonid diets to reduce phosphorus concentrations in hatchery effluents. The Prog. Fish-Cult: 57, 114–119. Doi: https://doi.org/10.1577/1548-8640(1995)057<0114:POSMWP>2.3.CO;2

CAO, L., WANG, W., YANG, C., YANG, Y., DIANA, J., YAKUPITIYAGE, A., LUO, Z. & LI, D. (2007). Application of microbial phytase in fish feed. J. Enz. Microb. Tech.: 40(4), 497–507. Doi: https://doi.org/10.1016/j.enzmictec.2007.01.007

CIAN, R.E., BACCHETTA, C., CAZENAVE, J. & DRAGO, S. (2018). Extruded fish feed with high residual phytase activity and low mineral leaching increased P. mesopotamicus mineral retention. Anim. Feed Sci. Tech.: 240, 78–87. Doi: https://doi.org/10.1016/j.anifeedsci.2018.03.016

DEAK, N.A. & JOHNSON, L.A. (2007). Fate of phytic acid in producing soy protein ingredients. J. Amer. Oil Chem. Soc.: 84(369), 369–376. Doi: https://doi.org/10.1007/s11746-007-1050-8

DEBNATH, D., PAL, A.K., SAHU, N.P., JAIN, K.K., YENGKOKPAM, S. & MUKHERJEE, S. (2005). Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of Pangasius pangasius fingerlings. Aquacul. Res.: 36(2), 180-187. Doi: https://doi.org/10.1111/j.1365-2109.2004.01203.x

DESCHAMPS, M-H., STEWART, N.P., DEMANCHE, A. & VANDENBERG, G.W. (2016). Preliminary study for a description of bone tissue responsiveness to prolonged dietary phosphorus deficiency in rainbow trout, Oncorhynchus mykiss (Walbaum). Aquacul. Res.: 47(3), 900-911. Doi: https://doi.org/10.1111/are.12549

DREW, M.D., RACZ, V.J., GAUTHIER, R. & THIESSEN, D.L. (2005). Effect of adding protease to coextruded flax: Pea or canola: Pea products on nutrient digestibility and growth performance of rainbow trout (Oncorhynchus mykiss). Anim. Feed Sci. Tech.: 119(1-2), 117–128 . Doi: https://doi.org/10.1016/j.anifeedsci.2004.10.010

EVANS, D.H., PIERMARINI, P.M. & CHOE, K.P. (2005). The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev.: 85, 97-177. Doi: https://doi.org/10.1152/physrev.00050.2003

FRANÇA, W.G.; BALLESTER, E.L.C. & PORTZ, L. Avaliação do uso da fitase em dietas à base de fontes vegetais na alimentação do pacu (Piaractus mesopotamicus). (Dissertation). Universidade Federal do Paraná, 47 p.

FURUYA, W.M., MICHELATO, M., SILVA, L.C.R., SANTOS, L.D., SILVA, T.S.C., SCHAMBER, C.R., VIDAL, L.V.O. & FURUYA, V.R.B. (2008). Fitase em rações de juvenis de pacu (Piaractus mesopotamicus). Bol. Inst. Pesca.: 34(4), 489–496.

FURUYA, W.M., SANTOS, V.G., BOTARO, D., HAYASHI, C. & SILVA, L.C.R. (2005). Níveis de proteína e fitase em rações de terminação para a tilápia do Nilo (Oreocrhomis niloticus). Arq. Ciênc. Vet. Zool.: 8(1), 11–17.

GREILING, A.M., TSCHESCHE, C., BAARDSEN, G., KRÖCKEL, S., KOPPE, W. & RODEHUTSCORD, M. (2019). Effects of phosphate and phytase supplementation on phytate degradation in rainbow trout (Oncorhynchus mykiss W.) and Atlantic salmon (Salmo salar L.). Aquaculture: 503, 467–474. Doi: https://doi.org/10.1016/j.aquaculture.2019.01.035

HABIB, R.Z., AFZAL, M., SHAD, S.Z., FATIMA, M., BILAL, M. & HUSSAIN, S. (2018). Potential of phytase and citric acid treated canola meal-based diet to enhance the minerals digestibility in Labeo rohita fingerlings. Zool. Soc. Pak.: 50. Doi: http://dx.doi.org/10.17582/journal.pjz/2018.50.6.2045.2050

HISCOCKS, S.A.; LUPATSCH, I., GOMES, G. & BEDFORD, M. (2020). An advanced Escherichia coli phytase improves performance and retention of phosphorus and nitrogen in rainbow trout (Oncorhynchus mykiss) fed low phosphorus plant-based diets, at 11 ºC and 15 ºC,

Aquaculture, 516, 734549. Doi: https://doi.org/10.1016/j.aquaculture.2019.734549

JACOBSEN, C. & BORRESEN, T. (1995). Formulation of fish diets with reduced phosphorous content. Water Sci. Tech.: 31(10), 167-173. Doi: https://doi.org/10.1016/0273-1223(95)00436-Q

JOBLING, M. (2012). National Research Council (NRC): Nutrient requirements of fish and shrimp. Aquacul. Internat.: 20, 601–602.

KHAJEPOUR, F., HOSSEINI, A. & IMANPOUR, M. (2012). Dietary crude protein, citric acid and microbial phytase and their interactions to influence growth performance, muscle proximate composition and hematocrit of common carp, Cyprinus carpio L, juveniles. World J Zool.: 7, 118–122.

KIM, K., PARK, Y., JE, H-W., SEONG, M., DAMUSARU, J.H., KIM, S., JUNG, J-Y. & BAI, S.C. (2019). Tuna byproducts as a fish meal in tilapia aquaculture. Ecotox. Environ. Safe.: 172(15), 364–372. Doi: https://doi.org/10.1016/j.ecoenv.2019.01.107

KUBITZA, F. (1999). Tanques-rede, rações e impacto ambiental. Rev. Panorama Aquicul.: 9, 44–50.

KUMAR, V., SINHA, A.K., MAKKAR, H., DE BOECK, G. & BECKER, K. (2012). Phytate and phytase in fish nutrition. J Anim. Physiol. Anim. Nut.: 96, 335–364. Doi: https://doi.org/10.1111/j.1439-0396.2011.01169.x

LALL, S.P. (1991). Digestibility, metabolism and excretion of dietary phosphorus in fish. In: Cowey CB and Cho, CY. Nutritional strategies and aquaculture waste. The University of Guelph.

LEMOS, D. & TACON, A.G.J. (2017). Use of phytases in fish and shrimp feeds: a review. Rev. Aquacul.: 9(3), 266–282. Doi: https://doi.org/10.1111/raq.12138

LI, M.H., ROBINETTE, R. & ROBINSON, E.H. (1996). Efficacy of dicalcium and defluorinated rock phosphates as dietary phosphorus sources for channel catfish (Ictalurus punctatus). Aquaculture: 147(1-2), 107–114. Doi: https://doi.org/10.1016/S0044-8486(96)01395-6

MAAS, R.M., VERDEGEM, M.C.J., DERSJANT-LI, Y. & SCHRAMA, J.W. (2018). The effect of phytase, xylanase and their combination on growth performance and nutrient utilization in Nile tilapia. Aquaculture: 487(25), 7–14. Doi: https://doi.org/10.1016/j.aquaculture.2017.12.040

MORALES, G.A., MÁRQUEZ, L., SAENZ DE RODRIGAÑEZ, M., BERMUDÉZ, L., ROBLES, R. & MOYANO, F.J. (2014). E ect of phytase supplementation of a plant-based diet on phosphorus and nitrogen bioavailability in sea bream Sparus aurata. Aquacul. Nutr.: 20(2), 172–182. Doi: https://doi.org/10.1111/anu.12063

NORAG, M.A.A., EL-SHENAWY, A., FADL, S., ABDO, W.S., GAD, D.M., RASHED, M.A. & PRINCE, A.M. (2018). Effect of phytase enzyme on growth performance, serum biochemical alteration, immune response and gene expression in Nile tilapia. Fish Shellfish Immunol.: 80, 97–108. Doi: https://doi.org/10.1016/j.fsi.2018.05.051

NWANNA, L.C., KOLAHSA, M., EISENREICH, R. & SCHWARZ, F. (2008). Pre-treatment of dietary plant feed stuffs with phytase and its effect on growth and mineral concentration in common carp (Cyprinus carpio). J. Anim. Physiol. Anim. Nutr.: 92(6), 677–682. Doi: https://

doi.org/10.1111/j.1439-0396.2007.00764.x

PEZZATO, L.E., BARROS, M.M. & FURUYA, W.M. (2009). Valor nutritivo dos alimentos utilizados na formulação de rações para peixes tropicais. R. Bras. Zootec.: 38, 43-51. Doi: http://dx.doi.org/10.1590/S1516-35982009001300005

RACHMAWATI, D. & SAMIDJAN, I. (2017). Performance efficiency of feed utilization, relative growth rate, and survival rate of common carp (Cyprinus carpio) through the addition of phytase in the feed. Conference Series-Earth and Environmental Science.: 137(01). Doi: https://doi.org/10.1088/1755-1315/137/1/012027

RODRIGUES, M.L., SANCHEZ, M.S.S., PESSINI, J.E., WEILER, K.A., DEPARIS, A., BOSCOLO, W.R., BITTENCOURT, F. & SIGNOR, A. (2020). Replacement of corn by sorghum and phytase supplementation in silver catfish (Rhamdia quelen) diets: growth performance, physiological variables and bone mineralization. J. Appl. Anim. Res.: 48(1), 142–150. Doi: https://doi.org/10.1080/09712119.2020.1750411

ROY, T., BANERJEE, G., DAN, S., GHOSH, P. & RAY, A. (2014). Improvement of nutritive value of sesame oilseed meal in formulated diets for rohu, Labeo rohita (Hamilton), fingerlings after fermentation with two phytase-producing bacterial strains isolated from fish gut. Aquacul. Internat.: 22, 633–652. Doi: https://doi.org/10.1007/s10499-013-9691-0

ROY, P.K. & LALL, S.P. (2003). Dietary phosphorus requirement of juvenile haddock (Melanogrammus aeglefinus L.). Aquaculture: 221(1-4), 451–468. Doi: https://doi.org/10.1016/S0044-8486(03)00065-6

SAJJADI, M. & CARTER, C. (2003). Dietary phytase supplementation and the utilization of phosphorus by Atlantic salmon (Salmo salar L.) fed a canola meal-based diet. The Asia Pacific. J. Clinic. Nutr.: 240(1-4), 417–431. Doi: https://doi.org/10.1016/j.aquaculture.2004.07.003

SAMIDJAN, I. & RACHMAWATI, D. (2017). Engineering technology of fish farming floating nets cages on Polka dot grouper (Cromileptes altivelis) used artificial feed enriched phytase enzyme. 3rd International Conference on Tropical and Coastal Region Eco-Development.: 116. Doi: https://doi.org/10.1088/1755-1315/116/1/012010

SAMPAIO, G.R., LOBÃO, V.L. & ROCCO, S.C. (2001). Uso de fosfatos como aditivos alimentares na redução de exsudato e nos atributos sensoriais da carne do camarão de água doce Macrobrachium rosenbergii. Bol. Instit. Pesca.: 27(1), 97–107.

SANTOS, K.O., COSTA-FILHO, J., SPAGNOL, K.L., NORNBERG, B.F., LOPES, F.M., TESSER, M.B. & MARINS, L.F. (2020). The inclusion of a transgenic probiotic expressing recombinant phytase in a diet with a high content of vegetable matter markedly improves growth performance and the expression of growth-related genes and other selected genes in zebrafish. Aquaculture, 519, 734878 Doi https://doi.org/10.1016/j.aquaculture.2019.734878

SHAH, S.Z.H., AFZAL, M., AKMAL, A., FATIMA, M. & HUSSAIN, S.M. (2016). Effect of citric acid and phytase on growth performance and mineralization of Labeo rohita juveniles fed soybean meal-based diet. Internat. J. Agricul. Biol.: 18(1), 111–116. Doi: https://doi.org/10.17957/IJAB/15.0071

SIGNOR, A.A., FEIDEN, A., BITTENCOURT, F., POTRICH, F.R., DEPARIS, A. & BOSCOLO, W.R. (2011). Fósforo na alimentação de pacu (Piaractus mesopotamicus). R. Bras. Zootec.: 40(12),

2646–2650. Doi: https://doi.org/10.1590/S1516-35982011001200005

SOUZA, L.W.O. (2004). Biodisponibilidade de fósforo em ingredientes alternativos – Revisão de literatura. Rev. Cient. Eletr. Med. Vet, 3, 1–4 ISSN 1679-7353.

WEILER, K.A., PESSINI, J.E., SANCHEZ, M., RODRIGUES, M., BOSCOLO, W., PEZZATO, L., BITTENCOURT, F. & SIGNOR, A. (2019). Sunflower meal with and without phytase supplementation in diets for silver catfish (Rhamdia queen) juveniles. R. Colomb. Ciênc. Pec.: 32, 285–297. Doi: https://doi.org/10.17533/udea.rccp.v32n4a04

YIGIT, N.O., KOCA, S.B., DIDINEN, B.I. & DILER, I. (2016). Effect of protease and phytase supplementation on growth performance and nutrient digestibility of rainbow trout (Oncorhynchus mykiss, Walbaum) fed soybean meal-based diets. J. Appl. Anim. Res.: 46, 1–4. Doi: https://doi.org/10.1080/09712119.2016.1256292

ZHENG, C., WU, J., JIN, Z., YE, Z. & FEI, H. (2019). Exogenous enzymes as functional additives in finfish aquaculture. Aquacul. Nutri.: 26, 213–224 Doi: https://doi.org/10.1111/anu.12995.

ZUGARKOVA, I., MARES, J., MALY, O. & GRMELA, J. (2018). Effect of phytase addition and citric acid on the production parameters of feed for Common Carp (Cyprinus carpio L.). Proceedings of 25th International Ph.D. Students Conference (MENDELNET), 185–188.