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Effect of phytase on digestibility of corn, sorghum and wheat bran by silver catfish (*Rhamdia voulezi*)

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ABSTRACT. This study aimed to evaluate the digestibility of plant energetic ingredients, corn, sorghum and wheat bran, with and without phytase supplementation, in silver catfish (*Rhamdia voulezi*). The experimental design was completely randomized, represented by eight treatments, which consisted of the development of a reference diet and a test diet for each ingredient evaluated (corn, sorghum and wheat bran), with and without supplemental phytase. Diets were formulated so as to contain 70% reference feed and 30% test ingredient. The digestibility coefficients of dry matter and crude protein were significantly influenced only in relation to the evaluated ingredients. Phytase influenced the digestibility coefficients of energy and phosphorus, with interaction between food and the enzyme addition. The interaction between these two factors shows that the phytase efficiency is dependent on the type of vegetable ingredient used in the diet. The results suggest the supplementation of phytase for a higher nutrient digestibility of corn, sorghum and wheat bran by silver catifish (*R. voulezi*).

Keywords: feeding, enzyme, plant energetic ingredients, silver catfish.

Efeito da fitase sobre a digestibilidade do milho, sorgo e farelo de trigo pelo jundiá (*Rhamdia voulezi*)

RESUMO. O objetivo do presente estudo foi avaliar a digestibilidade dos ingredientes energéticos vegetais, milho, sorgo e farelo de trigo, com e sem suplementação de fitase pelo jundiá (*Rhamdia voulezi*). O delineamento experimental foi inteiramente casualizado, representado por oito tratamentos, os quais consistiram na elaboração de uma dieta-referência e uma dieta- teste para cada ingrediente avaliado (milho, sorgo e farelo de trigo), com e sem a suplementação de fitase. As dietas foram fabricadas de modo que continham 70% da ração referência e 30% do ingrediente teste. Os coeficientes de digestibilidade da matéria seca e proteína bruta foram influenciados significativamente somente em relação aos ingredientes avaliados. A fitase influenciou nos coeficientes de digestibilidade da energia e do fósforo, onde também foi observada a interação entre os alimentos e a inclusão da enzima. A interação entre esses dois fatores demonstra que a eficiência da fitase é dependente do tipo de ingrediente vegetal utilizado na dieta. Pelos resultados obtidos sugere-se a suplementação de fitase visando maior digestibilidade dos nutrientes, do milho, sorgo e farelo de trigo pelo jundiá (*R. voulezi*).

Palavras-chave: alimentação, enzima, ingredientes energéticos vegetais, jundiá.

Introduction

Phytate or phytic acid is an anti-nutritional factor present in plant ingredients, formed by a myo-inositol ring containing six phosphate groups, hence its main function is the storage of phosphorus (National Research Council [NRC], 2011). It is a reactive anion regarded as the main anti-nutritional factor for the bioavailability of micronutrients (Brinch-Pedersen, Madsen, Holme, & Dionisio, 2014), and can form wide variety of insoluble salts with nutritionally important divalent cations, such as Ca^{2+}, Fe^{2+}, Zn^{2+}, in addition to complexing with proteins, lipids and starch (Kumar, Sinha, Makkar, De Boeck, & Becker, 2012).

Salts and complexes formed are not absorbed in the digestive tract of monogastric animals, including fish, since they cannot be solubilized at pH near that of the intestine (Greiner & Konietzny, 2006) affecting the variables of animal performance. This is because these animals do not have endogenous phytase in the gastrointestinal tract, which is responsible for gradual hydrolysis of the phytic acid molecule through a series of dephosphorylation reactions, resulting in a myo-inositol molecule and free phosphate (Sajjadi & Carter, 2004). By this hydrolysis, nutrients before complexed become available to be absorbed and utilized by the organism.

Scientific research related to the use of phytase in diets for fish has become increasingly significant, especially because it is considered an important and natural tool to combat pollution caused by residues from aquatic organisms (Brinch-Pedersen et al., 2014). When phytic phosphorus is made available for animal use, its supplementation in diet and its excretion into the aquatic environment are reduced.

Silver catfish is a species that has shown high potential for farming because of favorable performance characteristics for captive production, such as fast growth, easy adaptation to intensive farming and hardiness, combined with tasty meat, no intramuscular spines and with good acceptance by the market. Thus, the aim of this study was to evaluate the digestibility of plant energy ingredients, corn, sorghum and wheat bran with and without phytase supplementation by silver catfish (*Rhamdia voulezi*).

Material and methods

The digestibility analysis was conducted at the Aquaculture Laboratory of the Study Group on Management in Aquaculture – Gemaq, State University of Western Paraná, Unioeste, campus Toledo, in December 2013 and January 2014. Individuals of silver catfish (*R. voulezi*, n=288), with weight and length of 236.70 ± 54.02 g and 27.24 ± 1.79 cm, were stocked in 24 tanks of 500 L with tapered conical bottom, equipped with siphon system at the lower end for excreta collection. Fish were adapted to the experimental conditions for seven days.

The experimental design was completely randomized, with eight treatments and three replications. The treatments consisted of the development of a reference diet (Table 1) and a test diet for each evaluated ingredient (corn, sorghum and wheat bran), with and without supplemental phytase.

Diets were formulated so as to contain 70% of the reference feed and 30% of the ingredient tested. It was used 0.1% chromic oxide as inert marker mixed with the diet. The enzyme used (BASF-10000 FTU^{-1}) was produced from *Aspergillus niger* fungus and added to the diets supplemented in the amount of 1.500 FTU kg^{-1}, according to Rocha, Pouey, Enke, Xavier, and Almeida (2007).

The animals were fed twice a day (08h and 16h 30 min.) to satiation. Feces were collected daily, stored in plastic pots and kept in the freezer. At the end of the collection, the excreta were dried in forced ventilation oven at 55°C for 72h for laboratory analysis.

Table1. Reference diet.

Ingredients	Quantity (g kg^{-1})
Soybean meal	526.53
Corn grain	221.92
Broken rice	150.10
Poultry offal meal	30.00
Soybean oil	29.15
Fish meal	20.00
Premix-APP	10.00
Limestone	8.10
Salt	3.00
Antifungal	1.00
Antioxidant	0.20
Total	1000.00
Chemical composition	
Dry matter (%)	92.16
Crude protein (%)	31.92
Energy (kcal g^{-1})	4.32
Ether extract (%)	3.73
Mineral matter (%)	5.84

Samples of ingredients tested, feces and diets were analyzed for chemical composition and phosphorus in the laboratory of Food Quality Control, Unioeste. Physical and chemical analyses were made for crude protein, dry matter, mineral matter, ether extract and energy, according to (AOAC, 2005). In addition, diets and feces were subjected to analysis of chromic oxide, by atomic absorption reading, following the methodology described by Bremer Neto, Graner, Pezzato, Padovani e Cantelmo (2003). The results were used to calculate the digestibility coefficients of diets with the following formula:

$$ADC\ (\%) = 100.\ \{100\ x\ [(5\ Indicador_D/\ \%Indicador_F)\ x\ (N_F/N_D)]\}.$$

where ADC (%) is the digestibility coefficient of the diet; % $Indicator_D$ is the percentage of indicator in the diet; % $Indicator_F$ is the percentage of indicator in feces; N_F is the amount of nutrients present in feces and N_D is the amount of nutrients in the diet.

Then, the digestibility coefficients of ingredients tested were calculated:

$$ADC_{mg} = ADC\ (5)\ Dt + (ADC(\%)\ Dt - ADC\ (\%)\ Ref) \star [(b \star N_{mg})].$$

where ADC_{ing}: digestibility coefficient of the ingredient; ADC(%), Dt: digestibility coefficient of the test diet; ADC(%) Ref: digestibility coefficients of the reference diet; N_{ref}: amount of the nutrient in the reference diet; N_{ing}: the amount of nutrient in the ingredient tested; "b": percentage of the reference diet; "a": percentage of the test ingredient.

The observed digestibility coefficients were subjected to factorial analysis of variance ($p < 0.05$) and mean values were compared by Tukey's test. To

run these analyses, we used the software Statistica 7.0 (Statsoft, 2004).

Results and discussion

Data of chemical composition demonstrate that corn and sorghum are similar in nutrient composition (Table 2). Wheat bran presented higher values of crude protein, gross energy and phosphorus when compared to other ingredients tested. The chemical composition of ingredients observed in the present study corroborates the data described by Rostagno et al. (2011) and NRC (2011).

Table 2. Chemical composition of corn, sorghum and wheat bran used in digestibility evaluation by silver catfish (*R. voulezi*) (mean + standard deviation).

	Ingredients		
Chemical composition (%)	Corn	Sorghum	Wheat bran
Dry matter	89.76±0.53	88.72±0.89	89.46±0.30
Crude protein	8.91±0.36	8.85±0.28	17.46±0.66
Gross energy (kca kg^{-1})	3908.50±1.20	3966.50±1.53	4099.00±1.36
Ether extract	4.59±0.59	3.97±0.66	3.34±0.28
Phosphorus	0.26±0.01	0.30±0.00	0.47±0.01

The ADC of crude protein of corn found in the present study corroborates results obtained by Oliveira-Filho and Fracalossi (2006), who evaluated the digestibility of energy and protein feed for silver catfish (*Rhamdia quelen*) and observed ADC of protein of this ingredient of 83.6 ± 3.9. Regarding sorghum, Teixeira et al. (2010) registered ADC of dry matter and crude protein of 87.56 and 9.23% for juvenile surubim, lower than that obtained for protein in this study. Likewise, Rodrigues, Gominho-Rocha, Cargnin-Ferreira, Francisco, and Fracalossi (2012) verified lower values of ADC for these two parameters for silver catfish, being 36.0 and 58.7% for dry matter and crude protein, respectively.

The digestibility coefficients of dry matter and crude protein were significantly influenced ($p < 0.05$) only in relation to the evaluated ingredients. Sorghum showed a higher percentage of dry matter digestibility; 80.8% without the addition of phytase and 83.5% with the addition of phytase. Wheat bran stood out regarding the protein ADC 90.67% without addition of enzyme and 91.44% with the addition of the phytase, however, without significant difference from ADC of sorghum protein. Corn showed lower ADC values for both dry matter and for protein with and without addition of phytase (Table 3).

Phytase influenced the digestibility coefficients of energy and phosphorus, and it was also observed the interaction between the ingredients and the addition of the enzyme (Table 3). The interaction between these two factors show that the phytase efficiency is dependent on the type of vegetable ingredient used in the diet, considering that they contain phytase in the composition, and the amount is widely varied among the types of plants. For energy, it can be seen that corn without phytase has a lower percentage of ADC by *R. voulezi*, while sorghum with phytase stands out in relation to other ingredients, supplemented or not with the enzyme (Figure 1).

Only wheat bran caused no increase in the ADC of energy by the inclusion of the enzyme. This can be explained by the high content of phytase in composition of wheat and derivatives. According to Godoy, Chicco, Meschy, and Requena (2005), wheat bran has 2.173 FTU kg^{-1} phytase, which is higher than in corn and sorghum, with 24 and 35 FTU kg^{-1}, respectively. Thus, phytase in the ingredient may be inhibiting the effect of phytic acid on the chelation of nutrients, such as protein and starch.

Table 3. Digestibility coefficient of dry matter (DM), crude protein (CP), gross energy and phosphorus of corn, sorghum and wheat bran by silver catfish (*R. voulezi*).

				Ingredients		Probability	
Variables (%)	Phytase	Corn	Sorghum	Wheat bran	Phytase	Ingredients	Interactions
DM	Without	$76.50±3.6^{aA}$	$80.80±0.9^{bA}$	$75.90±1.6^{abA}$	0.20	0.00	0.21
	With	$75.30±0.9^{aA}$	$83.50±0.8^{bA}$	$77.90±1.8^{aA}$			
CP	Without	$84.50±1.2^{aA}$	$89.40±0.5^{bA}$	$90.67±0.4^{bA}$	0.44	0.00	0.28
	With	$84.50±3.4^{aA}$	$91.40±0.1^{bA}$	$91.44±0.8^{bA}$			
Energy gross	Without	$56.80±0.1^{aA}$	$83.06±0.2^{cA}$	$82.70±1.6^{bA}$	0.00	0.00	0.04
	With	$65.30±1.1^{aB}$	$86.16±0.4^{cB}$	$80.16±0.8^{bA}$			
Phosphorus	Without	$70.10±0.6^{aA}$	$64.20±2.3^{aA}$	$55.50±4.3^{bA}$	0.00	0.00	0.00
	With	$80.30±3.3^{aB}$	$71.40±1.8^{bcA}$	$75.90±2.5^{abB}$			
DP[1]	Without	7.50±0.1	7.90±0.1	15.80±0.1			
	With	7.40±0.3	8.10±0.0	15.90±0.1			
DE (kcal g^{-1})[2]	Without	2.94±0.0	3.29±0.1	3.28±0.6			
	With	3.05±0.0	3.41±0.1	3.30±0.3			

[1]DP= Digestible protein. [2]DE= Digestible energy. Values with different superscript lowercases, in the same row, are significantly different by Tukey's test ($p < 0.05$). Values with different superscript uppercases, in the same column, are significantly different by Tukey's test ($p < 0.05$).

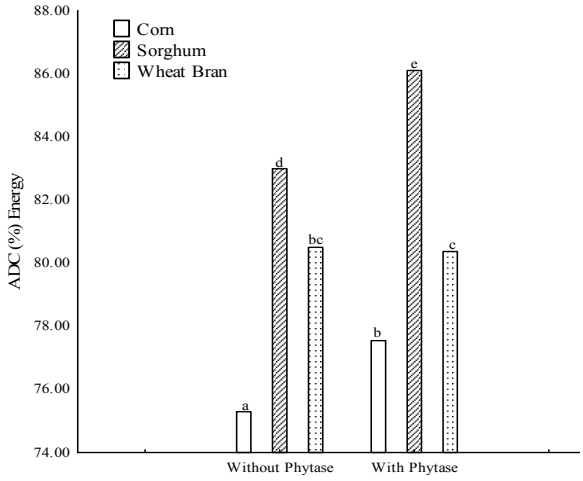

Figure 1. Apparent energy digestibility coefficient (ADC) of corn, sorghum and wheat bran with and without phytase by silver catfish (*Rhamdia voulezi*).

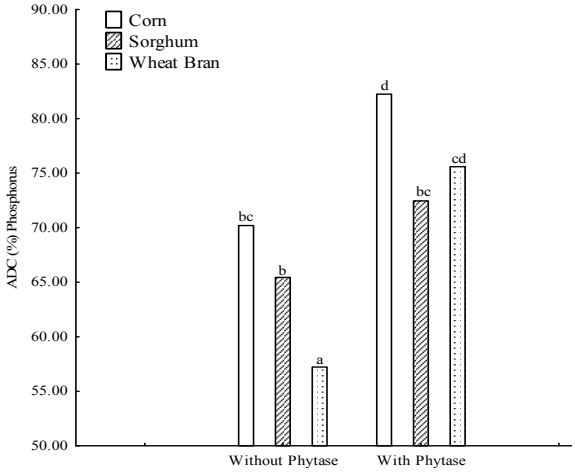

Figure 2. Apparent phosphorus digestibility coefficient (ADC) of corn, sorghum and wheat bran with and without phytase by silver catfish (*Rhamdia voulezi*).

The fact that phytase had provided increase in ADC of energy in sorghum and corn, in which there was 8.5% increase, demonstrates the effect of phytic acid in forming complexes and inhibiting nutrient absorption. Liu, Bold, Plumstead, and Selle (2015). claim that the phytase acts on the phytic acid molecule and promotes the dissociation of cations, such as sodium, resulting in greater efficiency in the absorption of molecules dependent on the sodium-potassium pump mechanism, like glucose, which is used as a source of energy in the metabolism and is primarily derived from energy food in the diet through the starch molecule.

Regarding the phosphorus, corn and wheat bran, supplemented with phytase, showed higher digestibility coefficients (Figure 2). Phytase promoted increases of 10.2 and 20.4% of ADC of phosphorus for corn and wheat bran, respectively. These results evidence the role of phytase in making available phytic phosphorus for use by silver catfish (*R. voulezi*).

The reduced phosphorus excretion is considered a key element for the sustainability of aquaculture in the long term (Morales, Moyano, and Marquez, 2011) and the use of nutritional strategies to reduce the excretion of this mineral into the water are among the most efficient methods (Bhavsar et al., 2013). Taking into consideration that the ingredients tested in this experiment are often used as energy sources in fish feed, it is evident the importance of using phytase to reduce phosphorus load through increased absorption and utilization in systems for rearing silver catfish (*R. voulezi*).

Conclusion

Phytase supplementation at 1.500 FTU kg^{-1} increases the digestibility coefficient of energy for corn and sorghum and of phosphorus for corn and wheat bran by silver catfish (*Rhamdia voulezi*).

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