Phytase on the digestibility of plant protein feed for silver catfish, Rhamdia voulezi

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ABSTRACT

This study evaluated the effect of diet supplementation with phytase enzyme (1,500 IU kg^{-1} of phytase) on the apparent digestibility parameters, availability and mineral deposits in the bones, intestinal histology, and proximate analysis on silver catfish (*Rhamdia voulezi*). A total of 360 silver catfish with an average weight of 236.98 ± 54.02 g were randomly distributed in 24–500 L conical-cylinder aquariums. The experimental design was completely randomized, represented by eight treatments and three replications in a factorial design. The eight treatments included formulations based on three different plant protein sources (soybean meal, canola meal, and sunflower meal) and one reference diet, each set differentiated by supplementation or no supplementation with phytase. The best CDA values and availability of nutrients were identified in the diet containing soybean meal. The reference diet without phytase resulted in increased intestinal villus height, and the diet containing canola meal without phytase resulted in thicker intestine muscular layers. The proximate compositions between diets with and without phytase showed significant differences ($p < 0.05$); the different protein sources responded in different ways to the activity of phytase. The diet with soybean meal showed the highest nutrient digestibility, and phytase supplementation improved the availability of dietary calcium and magnesium. The inclusion of sunflower meal (30%) caused harmful changes in the intestinal villi of the *Rhamdia voulezi*.

1. Introduction

Plant protein sources are essentially cheaper and available in higher quantities compared to fishmeal (Bergamin et al., 2013). In Brazil, soybean, canola, and sunflower have been produced in large scales for the production of edible oil and fuel (da Silva and de Freitas, 2008), which has increased the availability of these meals. However, the utilization of plant ingredients in animal feed is restricted by the presence of antinutritional substances that can act directly or through their metabolic products (Kumar et al., 2011; Chen et al., 2018). Phytates are among these antinutritional factors, they are of low availability and present in the concentrations of 0.34, 0.54, and 0.69% in soybean, canola, and sunflower meals, respectively (Rostagno et al., 2011; Surek et al., 2008).

Part of the phosphates present in plant ingredients are in the form of organic phosphorus, such as phytic phosphate (Riche and Brown, 1996) and have the ability to form complexes with cations, proteins, lipids, and starch (Szkudelski, 1998; Helland et al., 2006). However, according

to Vielma et al. (1998), the majority of these nutrients are not released when used as fish feed because fish intestines do not have the phytase enzyme, which is a phosphatase that breaks down the phytic phosphate molecule and improves nutrient availability for fish (Cao et al., 2007).

Phytase can be found naturally in plants and microorganisms and can be synthesized by fungi, yeast, and bacteria; animals do not synthesize phytase. Animals catalyze the hydrolysis of phytic acid monoester phosphate (known as myo-inositol 1, 2, 3, 4, 5, 6-hexaphosphate), which results in the gradual formation of myoinositol pentaquis, tetrakis-, tris-, bis-, and monophosphate along with the release of inorganic phosphate (Hurley et al., 2002; Singh and Satyanarayana, 2014).

Phytase acts on the catabolism of phytate molecules, available phosphorus, and other minerals, and is involved in their absorption, metabolism, and bone retention (Moreira et al., 2009; Kumar et al., 2011) enhancing the use of these minerals by the organism, which reduces their discharge into the environment and decreases the processes of eutrophication in aquatic environments (Hussain et al., 2011;

Lewandowski et al., 2017). There are several studies on the use of phytase in fish diets, however, comparing its activity and influencing the availability of nutrients in different foods are insufficient and much doubt hangs over its functionality and action and mainly with native species.

Species of the Rhamdia genre are among species with potential for fish farming. The silver catfish *R. voulezi* is one of these species (Freitas et al., 2011). This species has a natural preference for deeper locations, such as lakes and sinkholes, preferably with calm waters. It is considered by many authors as an omnivorous species with a carnivorous trend (Moro et al., 2010) because it can be fed diets that contain plant sources of protein as their main constituents (Rodrigues et al., 2011). This species shows rapid growth in intensive cultivation systems and have excellent meat quality with low fat, few bones, good carcass yield, and good consumer acceptance (Signor et al., 2013).

This study evaluated the effect of diet supplementation with phytase enzyme on the apparent digestibility parameters, availability and mineral deposits in the bones, intestinal histology and proximate analysis on catfish (*Rhamdia voulezi*).

2. Materials and methods

2.1. Experimental design

The experiments used a total of 360 fish with an average weight of 236.98 ± 54.02 g and an average length of 27.39 ± 2.02 cm. The fish were distributed in 24–500 L conical-cylinder aquariums fitted with a glass collector at the bottom where fecal material was deposited. The feces collection method used was modified from Guelph (Pezzato et al., 2002). The experimental design was completely randomized in a factorial design, with eight treatments (four with 1500 IU kg^{-1} of phytase supplementation and four without this supplementation) and three replications. One conical tank with 15 fish was considered one experimental unit. The animals were anesthetized in water benzocaine solution (100 mg L^{-1}) and distributions of animals were conducted in a way to minimize handling stress. A circular polyethylene net (with 2.5 cm mesh and 75.0 cm × 78.0 cm) was installed inside each experimental unit to contain the fish. Fish were fed three times a day to near satiation at 8 am, 12 pm, and 6 pm during 65 days following Furuya et al. (2012) procedure. The feces collector was attached daily at night and removed in the morning of the following day. This trial was approved by the ethics committee for animals use in CEUA at the same institution under protocol No. 047/12.

All experimental units were interconnected in the same recirculation system with water filtration. The water recirculation system consisted of a fiberglass 1000 L tank installed with a 300-watt thermostat to maintain the water temperature at 24 °C. Water was pumped into the system using a high-pressure water pump.

2.2. Assessment of the intestinal pH

Five fish were randomly collected for the assessment of midgut pH in the beginning of the experiments. These fish ($\approx$237 g) were euthanized in water and ice (4 °C), intestines were removed, and the midgut was weighed, macerated in a mortar and pestle, and transferred to Falcon tubes with Milli-Q water in the 1:10 ratio of weight (g) and volume (mL). The supernatant was centrifuged at 3000 rpm, and pH measurements were taken and showed an intestinal pH of 6.90 ± 0.02.

2.3. Experimental diets

The apparent digestibility coefficients was determined with formulations based on three protein sources: soybean meal, canola meal, and sunflower meal (Table 1), and one reference diet (Table 2). Therefore, three experimental diets and one reference diet were evaluated comprising two sets, one set with phytase supplementation at

Table 1
Chemical composition of the studied plant protein sources (dry matter as the baseline)[a].

Item	Soybean meal	Canola meal	Sunflower meal
Dry matter (g kg^{-1})[b]	893.60	917.10	916.40
Crude protein (g kg^{-1})[b]	439.50	299.90	342.30
Gross energy (Kcal kg-1)[b]	4147.00	4947.50	4483.50
Ether extract (g kg^{-1})[b]	41.40	168.10	68.80
Ash (g kg^{-1})[b]	73.40	63.20	55.60
Total phosphorus (g kg^{-1})[b]	7.10	7.60	7.60
Calcium (g kg^{-1})[c]	8.80	6.00	9.80
Mangnesium (g kg^{-1})[c]	3.60	4.90	6.50
Manganese (g kg^{-1})[c]	0.05	0.05	0.04
Copper (g kg^{-1})[c]	0.04	0.02	0.05
Zinc (g kg^{-1})[c]	0.70	0.90	1.20
Iron (g kg^{-1})[c]	0.60	1.00	1.50

[a] Values determined in the Laboratório de Análise de Alimentos (LQA) - Grupo de Estudos de Manejo na Aquicultura - GEMAq-Unioeste, Toledo-PR.
[b] Values determined in the Departamento de Química e Bioquímica do Instituto de Biociências da UNESP, Botucatu/SP.
[c] Values of analysis performed in triplicate.

1500 IU kg^{-1} of phytase (Rocha et al., 2007) and one set without it. The experimental diets contained 30% of the testing ingredient (soybean meal, canola meal, or sunflower meal) and 70% of the reference diet; 0.2% chromium oxide III was used as an inert tracer (Table 2). The reference diet was applied to determine the digestibility of nutrients.

Phytase (BASF - Natuphos®) at the concentration of 10,000 FTU g^{-1}, was obtained by the fermentation of *Aspergillus niger*; it was granulated and added to the diets. One active phytase unit (FTU) is considered as the amount of phytase that releases inorganic phosphorus from sodium phytate in a solution at the concentration of 5.1 mmol L^{-1} at pH 5.5 in one-minute reaction at 37 °C (Kornegay, 1999).

The ingredients selected for the formulations were individually milled in a hammer grinder with a 0.5 mm mesh screen, weighed, and homogenized in an automatic "Y" mixer including mineral and vitamin supplements without phosphorus. Chromium oxide and phytase were pre-mixed and subsequently homogenized with the rest of the ingredients in the diet. The ground diet was moistened with 22% of water, extruded in a 3.0 mm matrix at 80 °C, and pellets were dried in a forced ventilation oven at 55 °C for 24 h. The extrusion was carried out in an EXTRUTEC brand extruder with the capacity of 10 kg h^{-1} at the Laboratório de Nutrição de Organismos Aquáticos at Gemaq/Unioeste in Paraná.

2.4. Water quality parameters

The average values of water temperature, pH, dissolved oxygen, and electrical conductivity were respectively kept at 23.30 ± 0.06 °C; 7.88 ± 0.5; 5.23 ± 0.50 mg L^{-1} and 13.68 ± 0.12 μs cm^{-1} in the experimental units. These parameters were monitored with a YSI Professional Plus Multiparameter Water Quality Meter device, maintaining the recommended values for catfish culture (Piedras et al., 2018; de Oliveira Nuñer and Maffezzolli, 2006; De Garcia et al., 2011; Bolner et al., 2014).

2.5. Digestibility analysis

The concentration of chromic oxide evaluated in the feeds and feces to determine the apparent digestibility coefficient was measured by atomic absorption spectrophotometry and according to the methodology described by Neto et al. (2005). The apparent digestibility coefficient of each ingredient was calculated based on chromium oxide and the nutrient content in the feed and feces, as recommended by the equations reported in Nose (1960):

Table 2

Composition and nutritional values of the experimental diets supplemented with phytase enzyme or without supplementation (g kg^{-1} of dry matter).[1][a]

Ingredients	Reference	Diets without phytase supplementation			Diets with phytase supplementation		
		Soybean meal	Canola meal	Sunflower meal	Soybean meal	Canola meal	Sunflower meal
Soybean meal (45%	526.53	368.57	368.57	368.57	368.57	368.57	368.57
Corn	221.92	155.34	155.34	155.34	155.34	155.34	155.34
Broken rice	150.10	105.07	105.07	105.07	105.07	105.07	105.07
Chicken meal	30.00	21.00	21.00	21.00	21.00	21.00	21.00
Soybean oil	29.15	20.40	20.40	20.40	20.40	20.40	20.40
Fish meal (55%B)	20.00	14.00	14.00	14.00	14.00	14.00	14.00
Supplement (min. e vit.)[a]	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Calcium carbonate	8.10	5.67	5.67	5.67	5.67	5.67	5.67
Salt	3.00	2.10	2.10	2.10	2.10	2.10	2.10
Antifungal	1.00	0.70	0.70	0.70	0.70	0.70	0.70
Antioxidant	0.20	0.14	0.14	0.14	0.14	0.14	0.14
Soybean meal	–	296.10	–	–	296.10	–	–
Canola meal	–	–	296.10	–	–	296.10	–
Sunflower meal	–	–	–	296.10	–	–	296.10
Chemical composition							
Dry matter[2]	929.10	924.30	935.20	946.90	934.00	940.50	944.40
Crude protein[2]	306.50	299.40	322.60	312.60	296.40	317.00	314.60
Ether extract[2]	40.70	52.00	92.60	64.60	54.70	90.80	60.50
Gross energy(Kcal kg^{-1})[2]	4239.0	4391.0	4633.50	4532.50	4382.50	4671.50	4518.00
Ash[2]	110.40	67.70	66.70	64.90	66.20	68.20	65.80
Phosphorus[3]	5.90	6.80	7.60	6.60	6.00	7.60	7.60
Calcium[3]	12.20	9.00	6.70	6.80	9.20	7.10	7.20
Magnesium[3]	2.90	2.80	3.90	3.80	3.30	4.10	4.30
Manganese[3]	0.45	0.09	0.13	0.08	0.10	0.12	0.10
Copper[3]	0.04	0.04	0.04	0.04	0.05	0.04	0.04
Zinc[3]	2.20	0.60	0.40	0.50	0.60	0.40	0.50
Iron[3]	0.70	0.20	0.30	0.20	0.20	0.20	0.20

[a] Assurance levels per kilogram of product: Vit. A 1,750,000 UI; Vit. D3 375,000 UI; Vit. 20,000 UI; Vit. K3, 500 mg; Vit. B1, 2000 mg; Vit. B2, 2500 mg; Vit. B6, 2500 mg; Vit. B12 5000 mg; Folic acid 625 mg; Calcium pantothenate, 7500 mg; Vit. C 37,500 mg; Biotin, 50 mg; Inositol 12,500 mg; Niacin, 8750 mg; Coline, 100,000 mg; Co, 50 mg; Cu, 1250 mg; Fe, 15,000 mg; I, 100 mg; Mn, 3750 mg; Se, 75 mg; Zn, 17,500 mg.

[1] Values determined in the Laboratório de Análise de Alimentos (LQA) - Grupo de Estudos de Manejo na Aquicultura - GEMAq-Unioeste, Toledo-PR.

[2] Values determined in the Departamento de Química e Bioquímica do Institudo de Biociências da UNESP, Botucatu/SP.

[3] Values of analysis performed in triplicate.

$$ADC = 100 - \left(100. \frac{\%Cr_2O_3 r}{\%Cr_2O_3 f} . \frac{N_f}{N_r} \right) \tag{1}$$

where: ADC = apparent digestibility coefficient; Cr_2O_3 r% = % of chromium oxide in the formulation; Cr_2O_3 f% = % chromium oxide in the feces; N_f = nutrients in the feces; N_r = nutrients in the formulation.

The determination of the apparent digestibility coefficients (ADc) in the evaluated ingredients was carried out using the equation described by Cho (1979).

$$ADc_{ing} = \frac{(DC_{tr} - b \times DC_{br})}{a} \tag{2}$$

Where: ADc_{ing} = apparent digestibility coefficient of the ingredient; DC_{tr} = apparent digestibility coefficient of the feed ingredient in the test; DC_{br} = basal diet apparent digestibility coefficient; b = percentage of the basal diet; a = percentage of the testing ingredient.

Digestible values were obtained with the following equation:

$$DCa = \frac{DCa_{ing} \times N_f}{100} \tag{3}$$

where: DCa = apparent availability coefficient; DCa_{ing} = ingredient apparent digestibility coefficient; N_f = nutrient in the testing feed.

2.6. Intestinal morphometry

To evaluate the morphometry of the intestinal mucosa, portions of the medium intestine were collected, approximately 5 cm in length, from six fish at the beginning of the study that constituted the initial sample; two additional fish per experimental unit were sampled at the end of the study, totaling six fish per treatment. The samples were placed in a polystyrene plate (longitudinally), washed with saline solution, fixed in a 10% formaldehyde buffered solution for 12 h, dehydrated in an ascending series of alcohol and diaphanized in xylol for later inclusion in paraffin, for obtaining semi-serial histological sections of 7 μm. The sections were stained using the Hematoxylin-Eosin (HE) method. Image capture was performed using a 80× objective. The morphometry of the intestinal mucosa was performed on 100 villi per animal and villus heights were measured, from the upper end of the villus to the beginning of the muscular layer and the thickness of the muscular layer (de Almeida et al., 2008).

2.7. Proximate and mineral analysis

Six fish were randomly collected at the beginning of the study for the initial carcass evaluation. At the end of the experiment, two fish per tank were collected for the final carcass evaluation, totaling six fish per treatment. These fish were euthanized in 250 mg L^{-1} benzocaine, and fillets and vertebrae were removed.

The evaluation of physical, chemical, and energy parameters was conducted in the difference diets, feces, and fillets at the Laboratório de Análises de Alimentos (LQA), UNIOESTE, Toledo at Paraná, and in accordance with protocols approved by the AOAC (2016). The dry matter content was calculated in samples exposed to an oven at 105 °C until they reached constant weight (Tecnal, model TE-394/2), ashes were evaluated by calcination at 550 °C (TRADELAB, model 200D TLA), and lipid content was assessed using a specific solvent (petroleum ether) extraction equipment for lipids (Tecnal TE-044-5/50 model). The crude protein content was determined by the Kjeldahl method using a digestion (Tecnal, TE-018 model) and distillation system (Tecnal, TE-0363 model). Gross Energy was determined using a calorimeter pump (IKA, C Básic 2000).

All mineral determinations were performed in the Departamento de Química e Bioquímica do Instituto de Biociências, UNESP, Botucatu at São Paulo. The concentration of minerals contained in the ingredients, feed, and feces, was determined via nitropercloric digestion for posterior quantification. Calcium, magnesium, manganese, copper, zinc, and iron were determined by Atomic Absorption Spectrometry (AAS) following the procedures recommended by the manufacturer (Shimadzu-Cookbook, 2002).

2.8. Statistical analysis

Data from the apparent digestibility coefficients of dry matter, crude protein, crude fat, raw energy, total phosphorus, calcium, magnesium, manganese, copper, zinc, and iron were tabulated and submitted for analyses ($p < 0.05$) the normality checking assumptions of the waste performed by the Shapiro-Wilks test and homoscedasticity variance using the Levene test and factorials analysis were applied to determine interactions and differences between treatments. The Tukey's test for multiple comparisons was used to identify the sources of variation detected by the factor analysis. The data of mineral deposition in bones, gut histology, and fillet proximate analyses, with or without phytase supplementation, were compared to the initial sample using the Dunnett test. All statistical analyses were performed using the R software (R Development Core Team, 2013).

3. Results

3.1. Apparent digestibility coefficient

The factorial analysis (ingredient vs feed supplemented with or without phytase) of the Apparent Digestibility Coefficient (CDA) of crude protein, dry matter, and crude fat showed a significant effect ($p < 0.05$) on the interaction effect between ingredients and phytase supplementation; the best results, were observed in fish fed soybean meal supplemented with phytase (Table 3).

The data breakdown showed that the CDA was influenced by the feed and not by the phytase enzyme supplementation. Therefore, the soybean meal diet showed the highest apparent digestibility values for dry matter, crude protein, and crude fat while the sunflower meal diet showed the worst CDA values for these nutrients, yet the greatest influence of phytase supplementation was on the CDAs of mineral matter, which demonstrates the importance of supplementing the phytase enzyme to improve the availability of these nutrients in diets (Table 3).

3.2. Apparent digestibility of minerals

With the exceptions of copper and manganese, all other minerals CDA showed the effect ($p < 0.05$) of phytase supplementation and feed (Table 4). The enzyme supplementation improved the CDA of calcium and magnesium phosphorus, zinc and iron. The best CDA values were observed in the diet with soybean meal (Table 4). The CDA of magnesium, zinc, and iron showed no differences ($p > 0.05$) between the feeds with canola and sunflower meals. In general, the best CDAs for minerals were observed for soybean meal compared to other canola meal and sunflower meal.

3.3. The chemical composition of fillets

Fish fed the reference diets and soybean meal without phytase supplementation showed protein reduction in the file in relation to the values observed initially (Table 5). All treatments showed a significant ($p < 0.05$) increase in file fat content in relation to the values observed initially except for those fed the reference diet and canola meal without phytase supplementation. Fish fed the reference diet without phytase supplementation had a lower concentration of mineral matter compared to their initial values. For moisture content, fish fed soybean meal diets with or without phytase supplementation, canola meal without phytase and the reference diet without phytase showed higher moisture content compared to their initial values. There were no differences ($p > 0.05$) in the bromatological composition of fish fed the different test ingredients with or without phytase supplementation.

3.4. Minerals in the bones

Fish fed the experimental diets, regardless of phytase supplementation, showed a reduction ($p < 0.05$) in Mg concentration within in their bones in relation to the initial samples. There were no differences in the deposition of minerals P and Mg in the bones of fish due to the test ingredients and the supplementation or not of the phytase enzyme (Table 6). Fish fed the diet containing sunflower meal without phytase had the lowest concentration Cu and Zn minerals in their bones. Fish fed diets with canola meal and sunflower meal supplemented with phytase had a higher concentration of zinc in their bones. Regarding Ca, it was observed that fish fed with the reference diets with or without phytase supplementation and with the diet containing canola meal supplemented with phytase showed lower concentration in the bones.

Ca - calcium; P - total phosphorus; Mg - magnesium; Cu - copper; Zn - zinc.

Table 3
Apparent digestibility coefficients of dry matter, crude protein, gross energy, and crude fat in *R. voulezi* fed diets with or without phytase supplementation (% dry matter was used as the baseline).

Variables*	Phytase	Levels of food inclusion			(p-value)		
		Sunflower meal	Canola meal	Soybean meal	P[7]	F[8]	I[9]
CE	A[5]	$68.01 \pm 0.29^{A, a, ns}$	$77.50 \pm 1.61^{B, b, ns}$	$93.56 \pm 0.90^{C, d, ns}$	0.00	0.00	0.03
	P[6]	$74.96 \pm 1.17^{A, b, ns}$	$85.00 \pm 1.31^{B, c, ns}$	$97.57 \pm 0.61^{C, e, ns}$			
CP	A	$66.03 \pm 0.15^{A, a, 1}$	$76.56 \pm 0.73^{B, c, 1}$	$92.65 \pm 0.19^{C, e, 1}$	0.00	0.00	0.00
	P	$71.38 \pm 0.90^{A, b, 2}$	$79.69 \pm 0.48^{B, d, 2}$	$96.67 \pm 0.13^{C, f, 2}$			
CF	A	$67.33 \pm 0.49^{A, a, 1}$	$74.56 \pm 0.48^{B, bc, 1}$	$83.14 \pm 2.18^{C, d, 1}$	0.00	0.00	0.00
	P	$71.85 \pm 0.26^{A, b, 2}$	$75.30 \pm 0.56^{A, c, 2}$	$90.59 \pm 1.38^{B, e, 2}$			
Ash	A	$33.99 \pm 0.92^{A, a, 1}$	$32.36 \pm 0.67^{A, a, 1}$	$40.23 \pm 2.05^{B, b, 1}$	0.00	0.00	0.00
	P	$47.44 \pm 0.95^{A, c, 2}$	$59.43 \pm 0.61^{B, d, 2}$	$59.26 \pm 1.48^{B, d, 2}$			
DM	A	$66.17 \pm 0.56^{A, a, 1}$	$76.14 \pm 0.16^{B, c, 1}$	$91.86 \pm 0.48^{C, e, 1}$	0.00	0.00	0.03
	P	$72.10 \pm 0.68^{A, b, 2}$	$79.27 \pm 0.63^{A, d, 2}$	$96.49 \pm 0.42^{C, f, 2}$			

DM = dry matter; CP = crude protein; CE = crude energy; CF = crude fat.

* Capital letters (A, B, C, and D) on the lines indicate statistical difference between the evaluated feeds. Lower case (a, b, c, d, e and f) indicates interaction between feeds and phytase. Numbers (1 and 2) in the columns indicate difference in the presence or absence of phytase. P[7] (Phytase), F[8] (Feed), I[9] (Interaction); A (absent), P (Present). ns - Non significant ($p > 0.05$).

Table 4

Apparent digestibility coefficients of calcium, total phosphorus, magnesium, zinc, and iron in *R. voulezi* fed diets with or without phytase supplementation (% dry matter was used as the baseline).

Variables*	Phytase	Levels of food inclusion			(*p*-value)		
		Sunflower meal	Canola meal	Soybean meal	P[7]	AI[8]	I[9]
Ca	A[5]	$72.45 \pm 1.22^{A,\,b,\,1}$	$72.41 \pm 1.18^{A,\,b,\,1}$	$65.35 \pm 0.71^{B,\,a,\,1}$	0	0	0
	P[6]	$78.03 \pm 0.99^{A,\,c,\,2}$	$79.67 \pm 1.38^{A,\,c,\,2}$	$78.68 \pm 0.66^{A,\,c,\,2}$			
P	A	$15.01 \pm 1.12^{A,\,a,\,1}$	$42.29 \pm 1.37^{B,\,c,\,1}$	$65.72 \pm 2.31^{C,\,d,\,1}$	0	0	0
	P	$19.90 \pm 1.04^{A,\,b,\,2}$	$65.82 \pm 1.59^{B,\,d,\,2}$	$75.19 \pm 1.16^{C,\,e,\,2}$			
Mg	A	$72.14 \pm 1.39^{A,\,a,\,1}$	$72.83 \pm 1.06^{A,\,ab,\,1}$	$75.48 \pm 1.16^{A,\,b,\,1}$	0	0	0
	P	$85.36 \pm 1.16^{B,\,d,\,2}$	$79.59 \pm 0.92^{A,\,c,\,2}$	$83.71 \pm 0.88^{B,\,d,\,2}$			
Zn	A	$45.85 \pm 0.53^{B,\,b,\,1}$	$41.32 \pm 0.90^{A,\,a,\,1}$	$60.33 \pm 1.10^{C,\,e,\,1}$	0	0	0.02
	P	$54.26 \pm 1.16^{B,\,d,\,2}$	$51.25 \pm 0.60^{A,\,c,\,2}$	$66.98 \pm 0.76^{C,\,f,\,2}$			
Fe	A	$61.29 \pm 0.71^{B,\,b,\,1}$	$50.55 \pm 0.44^{A,\,a,\,1}$	$76.55 \pm 0.86^{C,\,e,\,1}$	0	0	0
	P	$67.30 \pm 1.19^{A,\,d,\,2}$	$64.43 \pm 1.18^{A,\,c,\,2}$	$81.33 \pm 1.41^{B,\,f,\,2}$			
Cu	A	$83.05 \pm 0.81^{A,\,a,\,ns}$	$85.33 \pm 0.48^{A,\,bc,\,ns}$	$89.26 \pm 0.18^{B,\,d,\,ns}$	0	0	0.01
	P	$84.47 \pm 0.40^{A,\,b,\,ns}$	$86.23 \pm 0.31^{A,\,ca,\,ns}$	$91.97 \pm 0.16^{B,\,e,\,ns}$			
Mn	A	$88.45 \pm 0.12^{A,\,b,\,ns}$	$86.49 \pm 0.47^{A,\,a,\,ns}$	$95.20 \pm 0.11^{B,\,e,\,ns}$	0	0	0
	P	$89.50 \pm 0.25^{A,\,c,\,ns}$	$91.85 \pm 0.32^{A,\,d,\,ns}$	$95.52 \pm 0.32^{B,\,e,\,ns}$			

Ca - calcium; Pt - total phosphorus; Mg - magnesium; Mn - manganese; Cu - copper; Zn - zinc; Fe - iron.

  * Capital letters (A, B, C, and D) on the lines indicate statistical difference between the evaluated feeds. Lower case (a, b, c, d, e and f) indicates interaction between feeds and phytase. Numbers (1 and 2) in the columns indicate difference in the presence or absence of phytase. P[7] (Phytase), F[8] (Feed), I[9] (Interaction); A (absent), P (Present). ns - Non significant (p > 0.05).

Table 5

Average values of proximate analysis of catfishes fillets fed with diets supplemented with or without phytase enzyme.

Treatment	Proximate analysis (g kg^{-1})				
	CP	CF	Ash	Moisture	P
Initial sample	173.60	65.20	14.70	746.60	10.40
Reference diet without phytase	144.10^{*c}	69.80^{b}	1.05^{*b}	780.30^{*a}	10.20
Reference diet with phytase	171.50^{a}	84.30^{*a}	1.51^{a}	735.60^{ab}	10.60
Soybean meal diet without phytase	155.50^{*b}	32.00^{*d}	1.41^{a}	794.90^{*a}	10.50
Soybean meal diet with phytase	160.008^{b}	50.80^{*c}	1.64^{a}	770.20^{*a}	10.80
Canola meal diet without phytase	166.20^{ab}	96.90^{*a}	1.14^{ab}	723.50^{*b}	10.10
Canola meal diet with phytase	157.20^{b}	86.50^{*a}	1.49^{a}	740.90^{ab}	10.30
Sunflower meal diet without phytase	166.70^{ab}	67.50^{b}	1.50^{a}	747.60^{ab}	10.90
Sunflower meal diet with phytase	157.80^{b}	80.40^{*a}	1.30^{a}	751.70^{ab}	11.20
F valor	4.30^{*}	31.04^{*}	4.28^{*}	13.90^{*}	0.55^{ns}

* Averages in same columns indicate differences (p < 0.05) of the sample in relation to the control by the *Dunnett* test. Different letters in same columns indicate differences (p < 0.05) of the sample by the *Tukey's* test. ns - Non-significant. CP - Crude protein; CF - crude fat; MM - Ash; P - Total phosphorus.

Table 7

Vilo height and muscle layer thickness of the middle intestine of *R. voulezi*, fed with diets supplemented or not with the enzyme phytase.

Treatment	Medium gut	
	Vilos hight	Muscle layer thickness
Initial sample	4.30	2.35
Reference diet without phytase	4.41	1.91*
Reference diet with phytase	6.24*	1.98*
Soybean meal diet without phytase	4.90*	2.05*
Soybean meal diet with phytase	6.10*	2.27
Canola meal diet without phytase	6.13*	3.16*
Canola meal diet with phytase	4.76*	1.65*
Sunflower meal diet without phytase	Not	Not
Sunflower meal diet with phytase	Not	Not
P valor	< 0.01	< 0.01
F valor	116.97	81.25

Means in the same column indicate differences (p < 0.05) between the variables analyzed by the Dunnett test.

Table 6

Vertebras averages values of calcium, total phosphorus, magnesium, copper and zinc in the vertebrae of catfish fed with diets supplemented with or without enzyme phytase.

Treatment	Proximate analysis of minerals in the vertebrae (mg kg^{-1})				
	Ca	P	Mg	Cu	Zn
Initial sample	278.00	196.10	36.00	18.60	113.90
Reference diet without phytase	238.60^{*c}	189.80	33.00^{*}	21.86^{*a}	116.37^{ab}
Reference diet with phytase	256.00^{*b}	199.00	31.00^{*}	20.60^{a}	117.82^{ab}
Soybean meal diet without phytase	275.90^{ab}	196.40	29.00^{*}	21.64^{*a}	92.88^{c}
Soybean meal diet with phytase	292.80^{a}	197.90	33.00^{*}	21.99^{*a}	102.18^{bc}
Canola meal diet without phytase	265.011^{b}	191.00	32.00^{*}	21.43^{*a}	105.88^{b}
Canola meal diet with phytase	249.80^{*bc}	199.30	32.00^{*}	22.97^{*a}	141.93^{*a}
Sunflower meal diet without phytase	273.80^{ab}	194.10	28.00^{*}	17.57^{b}	99.69^{c}
Sunflower meal diet with phytase	286.20^{a}	197.80	31.00^{*}	22.67^{*a}	136.39^{*a}
P valor	0.01	0.71	0.01	0.01	0.01
F valor	11.90^{*}	0.67	14.05	7.32^{*}	9.30^{*}

Averages in same columns indicate differences (p < 0.05) of the sample by the *Dunnett* test. Different letters in same columns indicate differences (p < 0.05) of the sample by the *Tukey's* test.
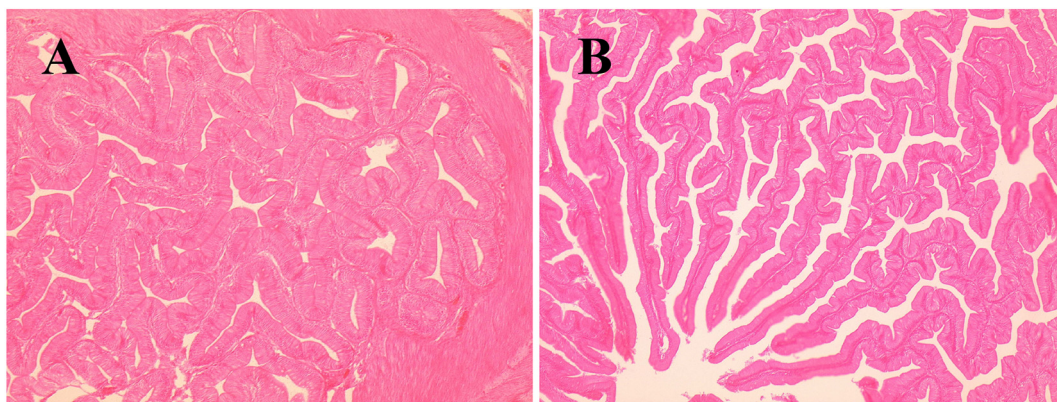
Fig. 1. Photomicrographs representative of the morphometric changes in the midgut of *R. voulezi* submitted to the sunflower diet with or without enzyme phytase. (A) sunflower without phytase supplementation and (B) sunflower with phytase supplementation. HE staining.

3.5. Intestinal histology

The greatest height of intestinal villi was observed in fish fed the reference diets containing phytase, fish fed with soy with phytase, and with canola without phytase supplementation (Table 7). Fish fed diets with canola meal with phytase and soybean meal without phytase had higher intestinal villus height averages than their initial values and the reference diet without phytase but did not differ from the others. It was not possible to measure the intestinal villus height of fish fed the sunflower meal diet with or without phytase enzyme supplementation, as the villi were contorted, and the measurements were totally compromised (Table 7; Fig. 1). Fish fed diets with canola meal supplemented with phytase and soybean meal without phytase had larger villi compared to the values observed initially, but smaller ($p < 0.05$) than those highlighted above. The greater thickness of the muscle layer was observed in fish fed diets containing canola meal without phytase (Table 7). Fish fed the reference diets with or without phytase supplementation and with canola meal without phytase supplementation had the lowest thickness of the intestinal muscle layer.

4. Discussion

4.1. Apparent digestibility coefficient

The intestinal pH of *R. voulezi* showed a mean of 6.9, which is expected for the species and this pH enhances the action of the phytase enzyme since the optimal pH of phytase action is between 6.5 and 7.5 (Konietzny and Greiner, 2002; Greiner and Konietzny, 2010). The CDA of dry matter of plant-based feeds obtained in this study are higher than those reported in catfish (*R. quelen*) by de Oliveira Filho and Fracalossi (2006) in a study evaluating the digestibility of soybean meal obtained 73.3% feed digestibility. The positive action of phytase enzyme supplementation in diets improving nutrient availability for fish has been demonstrated in several studies (Debnath et al., 2005a; Baruah et al., 2007; Lewandowski et al., 2017) and in the present study can inferred that phytase improves the availability of dietary nutrients for *R. quelen*. The CDA of dry matter provide an estimate for the total digestibility of the testing feed; generally, low values indicate that a large amount of non-digestible material is present (Li et al., 2011; Lewandowski et al., 2017). Thus, the CDA of dry matter estimate the amount of solid waste going into the environment and can be used to assess the environmental impact of an aquaculture production system.

The energy digestibility values for soybean meal found in this study were higher than those obtained by de Oliveira Filho and Fracalossi (2006) as 76.5% for juvenile catfish (*R. quelen*), by Zhou and Yue (2011) as 73.7% for hybrid tilapia, and by Kitagima and Fracalossi (2011) and Li et al. (2011) between 52.2 and 72.03% for channel catfish. However, the energy digestibility of canola meal (77.2%) reported

by Zhou and Yue (2011) in hybrid tilapia was similar to the values observed in our study. The differences observed in relation to the efficiency of phytase, in improving the digestibility of feed nutrients and even its action how the effect of the used levels, are linked to the biological value of these feeds, the nature and amount of phytic acid that these may present Gonçalves et al., 2004; Kumar et al., 2011; Hussain et al., 2011), and the crude fiber content present in the feeds. In the present study, it can be clearly seen that the CDAs of all evaluated nutrients were influenced by the type of feed and phytase supplementation, independently of the action of the phytase enzyme having important functional actions in the availability of nutrients in the diets, the composition of the feed can influence such actions. Sunflower and canola meal provided the lowest digestibility results observed for *R. voulezi* juveniles. Fibers, in turn, have an influence on the apparent digestibility of nutrients in diets, according to their utilization rate by interfering in gastric emptying time and acting in motility and intestinal movement by affecting the activity of digestive enzymes. In addition, fibers capture lipids micelles and interact with the surface of the intestinal wall influencing the absorption of nutrients (Madar and Thorne, 1987; Lanna et al., 2004).

An interaction between phytase supplementation and feed ingredient regarding lipid digestibility was observed in this study. Contrary effect was reported by Wang et al. (2008), who reported a reduction in lipid digestibility with phytase supplementation. The negative effect of phytase supplementation may be associated with the capacity of this enzyme to inhibit the activity of the lipase enzyme in competition for the substrate, decreasing the hydrolysis of lipids by lipase and thus causing a reduction in the absorption of these nutrients by animals (Wang et al., 2008; Liu et al., 2010). However, the improvement of fish digestibility of lipids due to phytase supplementation is intriguing, given the interaction that occurred between phytase supplementation and the diets with different feeds. It may be that between species there are interferences in the metabolic mechanism that lead to different metabolic behaviors and deserves further studies to explain these interactions.

The differences observed in the nutrient digestibility values are associated with variations of multiple factors, including the protein quality of feed used in rations, pH in the fish's stomach, and feed processing and procedures used during ration drying stages (Wang et al., 2008; Kumar et al., 2011). Moreover, the influence of phytase supplementation on nutrient digestibility also depends on several other factors in the diet such as the concentration and source of phytate, amount and source of protein, digestibility of protein source, and calcium and phosphorus levels (Sugiura et al., 2001; Singh, 2008; Chen et al., 2018). In this study, we used the phytase concentrations indicated for the species by Rocha et al. (2007) and the results indicate that the nutritional composition, especially the amount of phytate in the feed directly influences the phytase enzyme concentrations and activity. These

conditions deserve attention and should be better studied so that the enzyme is used as efficiently as possible.

The addition of phytase in diets with large amounts of plant-based protein significantly increases the digestibility of nutrients and reduce their level in feces (Sajjadi and Carter, 2004; Kumar et al., 2011; Lewandowski et al., 2017). Thus, the use of phytase in fish diets may be a powerful tool to reduce the release of nutrients into the aquatic environment, resulting in less water pollution since it promotes better nutrient absorption. The differences in the results reported in the literature and those observed in the present study may reflect the concentrations of phytate and phytase in the diets, the fish species studied, their eating habits, their stages of development, in addition to the possible constitution of a complex of antinutritional factors that these feeds may present in their composition.

4.2. Apparent digestibility of minerals

Approximately 70% of the total phosphorus from oleaginous seeds, cereal grains, and derivatives are present in the form of phytate, with low availability for fish (NRC, 2011). The low availability of phosphorus found in plant-based feeds is due to how this mineral is present in these ingredients (Gonçalves et al., 2007; Signor et al., 2016). In plants, phosphorus is part of inositol hexaphosphate or phytate molecules and may contain up to 81.0% of the phosphate content present in the plant (Riche and Brown, 1996); in addition, phytate presents high chelating ability with other minerals (Liu et al., 1998; Kumar et al., 2011) which impairs availability and compromising the nutrient absorption by the fish.

The results of this study indicate that the supplementation of 1500 IU kg^{-1} of phytase improves the digestibility of phosphorus, zinc, calcium, iron, and magnesium in catfish, showing a positive effect in these minerals. This condition was also observed by Lewandowski et al. (2017), when evaluating the effect of phytase supplementation (1500 IU of kg^{-1} of phytase) on the digestibility coefficients of oats, rice grits, and wheat middlings in silver catfish. Soybean meal and canola meal showed better availability of phosphorus compared to sunflower meal, this condition may be related to protein content, antinutritional factors, fiber content, and complexation of nutrients in sunflower meal; which consequently resulted in impairing the intestinal physiology of fish (Fig. 1), as well as the protein content linked to this mineral (Suttle, 2010; NRC, 2011). The results of this study, corroborate with the results reported by Gonçalves et al. (2005), in a study with Nile tilapia (*O. niloticus*) juveniles.

Phytase enzyme acts by hydrolyzing the phosphate-inositol bonds present in phytic acid, releasing inorganic phosphate and consequently releasing the minerals attached to this complex (Kumar and Sinha, 2018). In a study conducted by Chen et al. (2018) supplementing phytase levels in Channel Catfish diets, they reported the CDAs improvement of nutrients and minerals with the supplementation of 1500 FTU kg^{-1} of phytase. (Sugiura et al. (2001) observed an increase in copper absorption with the supplementation of phytase in a diet for rainbow trout. Debnath et al. (2005b) showed in *P. pangasius* fingerlings, that phytase supplementation of 500 FTU kg^{-1} is sufficient to increase the absorption of manganese. In the present study, no differences were observed in the CDAs of copper and manganese due to the supplementation of the enzyme phytase in the diets of *R. voulezi* is probably related to the presence of a large amount of fibers contents in the feed. However, it is observed that supplementation of phytase in the diets improved the availability of all other nutrients evaluated. Conditions that indicate the importance of phytase supplementation in diets for *R. voulezi*.

The availability coefficients of phosphorus were low in fish fed diets containing sunflower meal with or without phytase supplementation compared to other feeds. These results indicate the influence of phytate present in feeds that have lower concentrations of phytate and fiber (soybean meal and canola meal). The presence of large amounts of

antinutritional factors, it may compromise the functionality in releasing chelated nutrients and/or requiring a higher concentration of the enzyme in the diets so that the CDA of phosphorus for *R. voulezi* is improved. (Champagne, 1989). These results are corroborated by the observations reported by Graf (1983), highlighting the low phosphorus availability results from the quantitative precipitation of this mineral in the presence of phytate and acidic pH.

4.3. The chemical composition of fillets

Nutrients deficiency leads to biochemical disorder in organisms (Halver, 2002). The use of additives, such as enzymes, promote the availability of nutrients in diets. In the present study, the inclusion of phytase in fish diets provided greater availability of nutrients in the diets (see Tables 3 and 4).

The lipid and protein content of fish filled fed diets supplemented with phytase was improved over diets without phytase (Table 5), because the fish had the ability to store nutrients from the diets in their tissues (von Danwitz et al., 2016). However, the ash and phosphorus content in the fish was not affected by the inclusion of feed type or by supplementation of phytase in the diets.

4.4. Mineral in the bones

Our results in phosphorus retention in the vertebrae differ from results reported by Debnath et al. (2005c) in a study conducted with *Pangasius pangasius* juveniles fed diets with 350 FTU of phytase kg^{-1} where the autors reported the best calcium deposition and using 500 FTU of phytase kg^{-1} observed that the phytase supplementation increased the deposition of magnesium. The differences compared to our results might be related to the ingredients used, which provided different amounts of these nutrients in the diets. Phytase concentrations in the diet, fish development stages and moreover, between the studies and the species were also different between the studies. Therefore, the phosphorus status is very important for bone mineralization in fish. However, in the present study, we did not observe differences in phosphorus deposition in fish fed with different diets, which may be related to the phosphorus concentration of diets meeting fish nutritional requirements or the fact that a 65-day study period was not long enough for the fish to show any signs deficiency.

Given its importance in the formation of bone structure, an increase in phosphorus content in diets for fish leads to an increase in the level of phosphorus in bones causing an increase in the various mineral contents in the bones (Borlongan and Satoh, 2001; Zhang et al., 2006). Thus, demonstrating the importance of phytase supplementation in diets containing phytate to better make nutrients available for fish. Calcium and phosphorus are closely related to skeletal development and vertebrae stability (McDowell, 1985).

According to Porn Ngam et al. (1993), there is an increase in the inhibitory effects of calcium and phosphorus on zinc absorption when the calcium:phosphorus ratio goes far from 1:1 in the diet. Moreover, when there is an excess of calcium (high calcium:phosphorus ratio), this element may bind to the phytic phosphorus which, in turn, binds to zinc working as antagonist for ions of the same electrical charge and approximate size of zinc and magnesium (Henry et al., 2000; Suttle, 2010). However, if the calcium:phosphorus ratio is low because there is a phosphorus excess, the absorption of calcium and zinc decreases and in diets whose protein source is of plant origin and which contains high levels of phosphorus in the form of phytic acid will require phytase supplementation to better make its nutrients available to fish (Chen et al., 2018). In the present work Ca:P ratio was fixed at approximately 1:1 in the tested diets.

In this study, the results of zinc concentration in the vertebrae disagreed with those reported by Debnath et al. (2005c) in *P. pangasius* fingerlings; those authors did not find differences in zinc concentration in the vertebrae between the experimental and control groups when

using increasing levels of phytase in the diet. Factors such as levels of phytate, phosphorus, calcium, and protein source can inhibit or act competitively in the zinc-binding and absorption sites in fish (Gatlin and Wilson, 1984; Gatlin and Phillips, 1989; Vandenberg et al., 2011). Similar results were also reported by Yang et al. (2006) in "silver perch" juveniles and Helland et al. (2006) in Atlantic salmon, where an increase in phosphorus in the diet did not promote zinc increase in the vertebrae. According to Halver (2002), excess phosphorus in the diet can form chelate complexes with zinc and other trace elements, therefore, reducing phosphorus availability. Chen et al. (2018) observed that phytase supplementation in the diet increased the deposition of Ca, P, Mn, Zn and Cu in Channel catfish and that supplementation of 1500 FTU kg^{-1} provided the best results in weight gain and mineral deposition in the fish.

Debnath et al. (2005c) observed an improvement in a copper deposition in the vertebrae of *P. pangasius* with phytase supplementation. Sugiura et al. (2001) also observed an improvement in the copper vertebrae deposition of rainbow trout subjected to diets supplemented with phytase. In the present study, the lowest concentration of copper in the bones was observed in fish fed a diet containing sunflower meal without phytase supplementation and in relation to the control diet. The presence of antinutritional factors in sunflower meal may have influenced the reduced copper concentration in fish bones. When the diet containing sunflower meal is supplemented with phytase it acts on the availability of minerals through phytate molecule hydrolysis (Vandenberg et al., 2011).

4.5. Intestinal histology

Intestinal villi have a determining role in the absorption of nutrients from fish diets and the nutritional balance of diets is essential for animals to enhance their absorption. However, the presence of antinutritional factors in diets can compromise the gastrointestinal tract, impair digestibility and absorption, consequently affecting the productive performance and health of animals (Krogdahl et al., 2010; Martins et al., 2017). Among these antinutritional factors present in foods of plant origin, protease inhibitors, lecithins, saponins, phytates, tannins, glycosinolates, oligosaccharides, arginase and gossypol inhibitors (Francis et al., 2001; Selle et al., 2009; Bandara, 2018). The behavior presented by the intestinal villi of fish fed diets containing sunflower meal regardless of phytase supplementation status is an indication that antinutritional factors, such as phytic acid, arginase inhibitors, saponins and protease inhibitors are present in sunflower meal (Francis et al., 2001) may have acted negatively in the absorption of nutrients by the intestinal lumen and as a response of the organism conformational changes in the intestinal villi.

Still, on the behavior of the villi, it is assumed that this behavior of the intestinal villi of animals fed with a diet containing sunflower meal, is related to the amount of fibers present in this ingredient. The literature reports that the use of sources and levels of fibers in the nutrition of monogastric animals, influence the morphology of the intestinal mucosa, changing the height of the villi, depth of the crypts and the number of goblet cells (Jin et al., 1994; Klasing, 1998; Yu and Chiou, 1997). It can infer the favoring of the increase of intestinal villi in the present research. The scarcity of information in the scientific environment of sunflower meal on the intestinal morphology of fish makes it difficult to understand the villus behavior in the present study.

In diets formulated with sunflower meal, the presence of phytase is an important item to minimizing the impact of antinutritional factors present in this ingredient. The action of phytase on the degradation of phytic acid, an anti-nutritional factor that complexes minerals and proteins, (Forster et al., 1999; Tudkaew et al., 2008) such as phosphorus, copper, zinc, calcium, protein, and energy may have contributed to the results observed in relation to the greater height of the intestinal villi of fish. It is observed that the results indicate that there are interrelationships between the tested foods and phytase

supplementation on the availability of nutrients and the behavior of intestinal villi. Such results show that changes in the intestinal villi are the animal's responses to possible stress that the diet may cause and that the increase in the thickness of the muscular layer and height of the villi does not always correspond to the improvement in the absorption of nutrients. The fact that some species of fish show phytase activity in the intestine (Ellestad et al., 2002) may be related to its presence in their diet, for *R. voulezi* such activities are unknown and in the present study they were not evaluated. Due to the fact that foods present different concentrations of the anti-nutritional factors reported above, they could influence the activities of the phytase enzyme in the intestinal lumen of fish, which evidently demonstrates the importance of having considered this type of analysis in the present study.

One of the factors directly related to the results obtained is the use of the high levels of inclusion (30%) of the test ingredients, which probably interfered significantly in the observed responses regarding the behavior of intestinal villi and digestibility coefficients. Considering that the use of 30% inclusion of test ingredients and 70% of the reference diet is standard in food digestibility assessments (Bureau et al., 1999; Dong et al., 2010; NRC, 2011; Lewandowski et al., 2017),These results lead us to a reflection that such evaluations should consider the type of food to be evaluated and therefore research with this purpose should be done to better understand these interactions and how levels of inclusion can compromise the physiological capacity of the fish.

5. Conclusions

The soybean meal diet showed the highest nutrient digestibility value and the phytase supplementation improved the availability of dietary calcium and magnesium. The greatest height of intestinal villi was observed in fish fed with the reference diet supplemented with phytase, and the greatest thickness of muscular layers was observed in fish fed with the canola meal diet without phytase.

The results suggest that the phytase supplementation influences the Zn deposition in bones. The phytase supplementation did not affect the phosphorus and magnesium deposition in the vertebra and phosphorus and mineral matter in fish fillet; fish fed diets without phytase supplementation show higher crude fat concentration. The inclusion of sunflower meal (30%) in *R. voulezi* diets regardless of phytase supplementation caused harmful changes in the intestinal villi of the *Rhamdia voulezi*. The phytase activity in the availability of nutrients is directly related to the nutritional composition of foods to be used in the preparation of diets. Sunflower meal has low availability of its nutrients to *Rhamdia voulezi*.

Declaration of Competing Interest

None.

References

NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press https://doi.org/10.17226/13039.

de Almeida, F.L.A., Carvalho, R.F., Pinhal, D., Padovani, C.R., Martins, C., Pai-Silva, M.D., 2008. Differential expression of myogenic regulatory factor MyoD in pacu skeletal muscle (piaractus mesopotamicus holmberg 1887: Serrasalminae, characidae, teleostei) during juvenile and adult growth phases. Micron 39, 1306–1311. https://doi.org/10.1016/j.micron.2008.02.011.

AOAC, 2016. Official Methods of Analysis of the Association of Official Analytical Chemists, 20th ed. John Wiley & Sons, Inc, Hoboken, NJ, USA.

Bandara, T., 2018. Alternative feed ingredients in aquaculture: opportunities and challenges. Journal of Entomology and Zoology Studies 6, 3087–3094.

Baruah, K., Pal, A.K., Sahu, N.P., Debnath, D., Nourozitallab, P., Sorgeloos, P., 2007. Microbial phytase supplementation in rohu, labeo rohita, diets enhances growth performance and nutrient digestibility. Journal of the World Aquaculture Society 38, 129–137. https://doi.org/10.1111/j.1749-7345.2006.00081.x.

Bergamin, G.T., Veiverberg, C.A., Siqueira, L.V., Eggers, D.P., Neto, J.R., 2013. Digestibilidade aparente de farelos vegetais tratados para remoção de antinutrientes em dietas para jundiá. Pesq. Agrop. Brasileira 48, 928–934. https://doi.org/10.1590/s0100-204x2013000800017.

Bolner, K.C., Copatti, C.E., Rosso, F.L., Loro, V.L., Baldisserotto, B., 2014. Water pH and metabolic parameters in silver catfish (rhamdia quelen). Biochem. Syst. Ecol. 56, 202–208. https://doi.org/10.1016/j.bse.2014.06.006.

Borlongan, I.G., Satoh, S., 2001. Dietary phosphorus requirement of juvenile milkfish,-chanos chanos (forsskal). Aquac. Res. 32, 26–32. https://doi.org/10.1046/j.1355-557x.2001.00003.x.

Bureau, D., Harris, A., Cho, C., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (oncorhynchus mykiss). Aquaculture 180, 345–358. https://doi.org/10.1016/s0044-8486(99)00210-0.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z., Li, D., 2007. Application of microbial phytase in fish feed. Enzym. Microb. Technol. 40, 497–507. https://doi.org/10.1016/j.enzmictec.2007.01.007.

Champagne, E.T., 1989. Low gastric hydrochloric acid secretion and mineral bioavailability. In: Mineral absorption in the monogastric GI tract. Springer, pp. 173–184.

Chen, A., Liu, X., Cui, C., Yang, C., Wang, Y., Bu, X., Yang, Y., 2018. An evaluation of phytase for channel catfish (ictalurus punctatus) fed all plant-protein diet: growth performance, nutrient utilization and p equivalency value. Aquac. Nutr. 25, 215–224. https://doi.org/10.1111/anu.12845.

Cho, C. (1979). Apparent digestibility measurement in feedstuffs for rainbow trout. In *Proc. World Symp. on Finfish Nutrition and Fishfeed Technology Vol. II* (pp. 239–247).

von Danwitz, A., van Bussel, C.G., Klatt, S.F., Schulz, C., 2016. Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (psetta maxima l.). Aquaculture 450, 405–411. https://doi.org/10.1016/j.aquaculture.2015.07.026.

Debnath, D., Pal, A.K., Sahu, N.P., Jain, K.K., Yengkokpam, S., Mukherjee, S.C., 2005a. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of pangasius pangasius (Hamilton) fingerlings. Aquac. Res. 36, 180–187. https://doi.org/10.1111/j.1365-2109.2004.01203.x.

Debnath, D., Pal, A.K., Sahu, N.P., Jain, K.K., Yengkokpam, S., Mukherjee, S.C., 2005b. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of pangasius pangasius (Hamilton) fingerlings. Aquac. Res. 36, 180–187. https://doi.org/10.1111/j.1365-2109.2004.01203.x.

Debnath, D., Sahu, N.P., Pal, A.K., Jain, K.K., Yengkokpam, S., Mukherjee, S.C., 2005c. Mineral status of pangasius pangasius (Hamilton) fingerlings in relation to supplemental phytase: absorption, whole-body and bone mineral content. Aquac. Res. 36, 326–335. https://doi.org/10.1111/j.1365-2109.2004.01204.x.

R Development Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Dong, X.-H., Guo, Y.-X., Ye, J.-D., Song, W.-D., Huang, X.-H., Wang, H., 2010. Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, oreochromis niloticus × oreochromis aureus. Aquac. Res. 41, 1356–1364. https://doi.org/10.1111/j.1365-2109.2009.02424.x.

Ellestad, L., Angel, R., Soares, J., 2002. Intestinal phytase ii: a comparison of activity and in vivo phytate hydrolysis in three teleost species with differing digestive strategies. Fish Physiol. Biochem. 26, 259–273.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M., Parr, J., 1999. Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout (oncorhynchus mykiss) held in 11c fresh water. Aquaculture 179, 109–125. https://doi.org/10.1016/s0044-8486(99)00156-8.

Francis, G., Makkar, H.P., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227. https://doi.org/10.1016/s0044-8486(01)00526-9.

Freitas, J.M.A.D., Sary, C., Luchesi, J.D., Feiden, A., Boscolo, W.R., 2011. Protena e energia na dieta de jundiás criados em tanques-rede. Rev. Bras. Zootec. 40, 2628–2633.

Furuya, W.M., Graciano, T.S., Vidal, L.V.O., Xavier, T.O., Gongora, L.D., Righetti, J.S., Furuya, V.R.B., 2012. Digestible lysine requirement of nile tilapia fingerlings fed arginine-tolysine-balanced diets. Revista Brasileira de Zootecnia 41, 485–490. https://doi.org/10.1590/s1516-35982012000300003.

Gatlin, D.M., Phillips, H.F., 1989. Dietary calcium, phytate and zinc interactions in channel catfish. Aquaculture 79, 259–266. https://doi.org/10.1016/0044-8486(89)90466-3.

Gatlin, D.M., Wilson, R.P., 1984. Zinc supplementation of practical channel catfish diets. Aquaculture 41, 31–36. https://doi.org/10.1016/0044-8486(84)90387-9.

Gonçalves, G.S., Pezzato, L.E., Barros, M.M., Hisano, H., Freire, E.D.S., Ferrari, J.E.C., 2004. Digestibilidade aparente e suplementação de fitase em alimentos vegetais para tilápia do nilo. Acta Scientiarum. Animal Sciences 26. https://doi.org/10.4025/actascianimsci.v26i3.1795.

Gonçalves, G.S., Pezzato, L.E., Barros, M.M., Kleeman, G.K., Rocha, D.F., 2005. Efeitos da suplementação de fitase sobre a disponibilidade aparente de mg, ca, zn, cu, mn e fe em alimentos vegetais para a tilápia-do-nilo. Rev. Bras. Zootec. 34, 2155–2163. https://doi.org/10.1590/s1516-35982005000700001.

Gonçalves, G.S., Pezzato, L.E., de Magalhães Padilha, P., Barros, M.M., 2007. Disponibilidade aparente do fósforo em alimentos vegetais e suplementação da enzima fitase para tilápia-do-nilo. Rev. Bras. Zootec. 36, 1473–1480. https://doi.org/

10.1590/s1516-35982007000700003.

Graf, E., 1983. Applications of phytic acid. Journal of the American Oil Chemists Society 60, 1861–1867. https://doi.org/10.1007/bf02901539.

Greiner, R., Konietzny, U., 2010. Phytases: Biochemistry, enzymology and characteristics relevant to animal feed use. In: Enzymes in Farm Animal Nutrition. Wallingford, UK, CABI Publishing, pp. 96–128. https://doi.org/10.1079/9781845936747.0096.

Halver, J., 2002. The minerals. In: Fish Nutrition. Academic Press, pp. 61–141.

Helland, S., Denstadli, V., Witten, P.E., Hjelde, K., Storebakken, T., Skrede, A., Åsgård, T., Baeverfjord, G., 2006. Hyper dense vertebrae and mineral content in Atlantic salmon (salmo Salar l.) fed diets with graded levels of phytic acid. Aquaculture 261, 603–614. https://doi.org/10.1016/j.aquaculture.2006.08.027.

Henry, P.R., Miles, R.D., et al., 2000. Interactions among the trace minerals. Ciência Animal Brasileira 1, 95–106.

Hurley, L., Stanton, T., Jarosz, M., Schutz, D., 2002. Effects of dietary phosphorus and microbial phytase1 level on beef finishing performance1microbial phytase was provided by BASF, mount olive, NJ 07828-1234. The Professional Animal Scientist 18, 286–292. https://doi.org/10.15232/s1080-7446(15)31535-7.

Hussain, S.M., Afzal, M., Rana, S.A., Javid, A., Hussain, M., 2011. Impact of phytase supplementation on nutrient digestibility for labeo rohita fingerlings fed on sunflower meal based diets. Pak. J. Life Soc. Sci 9, 85–90.

Jin, J., Reynolds, L.P., Redmer, D.A., Caton, J.S., Crenshaw, J.D., 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs2. Journal of Animal Science 72, 2270–2278. https://doi.org/10.2527/1994.7292270x.

Kitagima, R.E., Fracalossi, D.M., 2011. Digestibility of alternative protein-rich feedstuffs for channel catfish, ictalurus punctatus. J. World Aquacult. Soc. 42, 306–312. https://doi.org/10.1111/j.1749-7345.2011.00468.x.

Klasing, K., 1998. Nutritional modulation of resistance to infectious diseases. Poult. Sci. 77, 1119–1125.

Konietzny, U., Greiner, R., 2002. Molecular and catalytic properties of phytate-degrading enzymes (phytases). International Journal of Food Science and Technology 37, 791–812. https://doi.org/10.1046/j.1365-2621.2002.00617.x.

Kornegay, E., 1999. Feeding to reduce nutrient excretion: effects of phytase on phosphorus and other nutrients. In: Lyons, T.P., Jacques, K.A. (Eds.), Biotechnology in the feed industry: Proceeding of Alltech's 15th Annual Symposium, pp. 461–489.

Krogdahl, Å., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important anti-nutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquac. Res. 41, 333–344. https://doi.org/10.1111/j.1365-2109.2009.02426.x.

Kumar, V., Sinha, A.K., 2018. General aspects of phytases. In: Enzymes in Human and Animal Nutrition. Elsevier, pp. 53–72. https://doi.org/10.1016/b978-0-12-805419-2.00003-4.

Kumar, V., Makkar, H., Becker, K., 2011. Detoxified jatropha curcas kernel meal as a dietary protein source: growth performance, nutrient utilization and digestive enzymes in common carp (cyprinus Carpio l.) fingerlings. Aquac. Nutr. 17, 313–326. https://doi.org/10.1111/j.1365-2095.2010.00777.x.

Lanna, E.A.T., Pezzato, L.E., Cecon, P.R., Furuya, W.M., Bomfim, M.A.D., 2004. Digestibilidade aparente e trânsito gastrintestinal em tilápia do nilo (oreochromis niloticus), em função da fibra bruta da dieta. Rev. Bras. Zootec. 33, 2186–2192. https://doi.org/10.1590/s1516-35982004000900002.

Lewandowski, V., Feiden, A., Signor, A., Bittencourt, F., Moro, E.B., Pessini, J.E., Boscolo, W.R., 2017. Digestibility of vegetal energetic ingredients supplemented with phytase for silver catfish (rhamdia voulezi). Aquaculture 467, 71–75. https://doi.org/10.1016/j.aquaculture.2016.05.022.

Li, M.H., Oberle, D.F., Lucas, P.M., 2011. Apparent digestibility of alternative plant-protein feedstuffs for channel catfish, *Ictalurus punctatus*(rafinesque). Aquac. Res. 44, 282–288. https://doi.org/10.1111/j.1365-2109.2011.03035.x.

Liu, B.-L., Rafiq, A., Tzeng, Y.-M., Rob, A., 1998. The induction and characterization of phytase and beyond. Enzym. Microb. Technol. 22, 415–424. https://doi.org/10.1016/s0141-0229(97)00210-x.

Liu, N., Ru, Y., Wang, J., Xu, T., 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. British Journal of Nutrition 103, 862–868. https://doi.org/10.1017/s0007114509992558.

Madar, Z., Thorne, R., 1987. Dietary fiber. Progress in food & nutrition science 11, 153–174.

Martins, G.P., Pezzato, L.E., Guimaraes, I.G., Padovani, C.R., Mazini, B.S.M., Barros, M.M., 2017. Antinutritional factors of raw soybean on growth and haematological responses of nile tilapia. Boletim do Instituto de Pesca 43, 322–333. https://doi.org/10.20950/1678-2305.2017v43n3p322.

McDowell, L., 1985. Iron, Manganese, and Zinc. Academic Press Orlando, FL.

Moreira, J., Lopes, J., Vitti, D., Teixeira, A., 2009. Modelos matemáticos para o estudo do fluxo biológico do fósforo em sunos alimentados com dietas suplementadas com nveis crescentes de fitase. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 61, 420–428. https://doi.org/10.1590/s0102-09352009000200020.

Moro, G.V., Camilo, R.Y., Moraes, G., Fracalossi, D.M., 2010. Dietary non-protein energy sources: growth, digestive enzyme activities and nutrient utilization by the catfish jundiá, *Rhamdia quelen*. Aquac. Res. 41, 394–400. https://doi.org/10.1111/j.1365-2109.2009.02352.x.

Neto, H.B., Graner, C.A.F., Pezzato, L.E., Padovani, C.R., 2005. Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado ao 1,5-difenilcarbazida. Ciência Rural 35, 691–697. https://doi.org/10.1590/s0103-84782005000300033.

Nose, T., 1960. On the digestion of food protein by gold-fish (carassius auratus l.) and rainbow trout (salmo irideus g.). Bulletin of Freshwater Fisheries Research Laboratory 10, 11–22.

De Garcia, L., Becker, A.G., Cunha, M.A., Baldisserotto, B., Copatti, C.E., Kochhann, D., 2011. Effects of water pH and hardness on infection of silver catfish, rhamdia quelen,

fingerlings by ichthyophthirius multifiliis. J. World Aquacult. Soc. 42, 399–405. https://doi.org/10.1111/j.1749-7345.2011.00479.x.

de Oliveira Filho, P.R.C., Fracalossi, D.M., 2006. Coeficientes de digestibilidade aparente de ingredientes para juvenis de jundiá. Rev. Bras. Zootec. 35, 1581–1587. https://doi.org/10.1590/s1516-35982006000600002.

de Oliveira Nuñer, A.P., Maffezzolli, G., 2006. Crescimento de alevinos de jundiá, rhamdia quelen (pisces, pimelodidae), em diferentes concentrações de oxigênio dissolvido. Acta Scientiarum. Biological Sciences 28. https://doi.org/10.4025/actascibiolsci.v28i1.1057.

Pezzato, L.E., de Miranda, E.C., Barros, M.M., Pinto, L.G.Q., Furuya, W.M., Pezzato, A.C., 2002. Digestibilidade aparente de ingredientes pela tilápia do nilo (oreochromis niloticus). Revista Brasileira de Zootecnia 31, 1595–1604. https://doi.org/10.1590/s1516-35982002000700001.

Piedras, S., Moraes, P., Pouey, J., 2018. Crescimento de juvenis de jundiá (rhamdia quelen), de acordo com a temperatura da água. Bol. Inst. Pesca 30, 177–182.

Porn Ngam, N., Satoh, S., Takeuchi, T., Watanabe, T., 1993. Effect of the ratio of phosphorus to calcium on zinc availability to rainbow trout [oncorhynchus mykiss] in high phosphorus diet. Bulletin of the Japanese Society of Scientific Fisheries (Japan) 59 (12), 2065–2070. https://doi.org/10.2331/suisan.59.2065.

Riche, M., Brown, P.B., 1996. Availability of phosphorus from feedstuffs fed to rainbow trout, oncorhynchus mykiss. Aquaculture 142, 269–282. https://doi.org/10.1016/0044-8486(95)01218-4.

Rocha, C.B., Pouey, J.L.O.F., Enke, D.B.S., Xavier, E.G., Almeida, D.B., 2007. Suplementação de fitase microbiana na dieta de alevinos de jundiá: efeito sobre o desempenho produtivo e as características de carcaça. Ciência Rural 37, 1772–1778. https://doi.org/10.1590/s0103-84782007000600042.

Rodrigues, A., Gominho-Rosa, M., Cargnin-Ferreira, E., Francisco, A.D., Fracalossi, D., 2011. Different utilization of plant sources by the omnivores jundiá catfish (rhamdia quelen) and nile tilapia (oreochromis niloticus). Aquac. Nutr. 18, 65–72. https://doi.org/10.1111/j.1365-2095.2011.00877.x.

Rostagno, H., Albino, L., Donzele, J., Gomes, P., De Oliveira, R., Lopes, D., Ferreira, A., Barreto, S., Euclides, R., 2011. Tabelas brasileiras para suínos e aves: composição de alimentos e exigências nutricionais (HS Rostagno, Ed.). 3rd ed UFV, Viçosa, Minas Gerais, Brasil.

Sajjadi, M., Carter, C., 2004. Effect of phytic acid and phytase on feed intake, growth, digestibility and trypsin activity in Atlantic salmon (salmo Salar, l.). Aquac. Nutr. 10, 135–142. https://doi.org/10.1111/j.1365-2095.2003.00290.x.

Shimadzu, Cookbook, 2002. Operation manual: atomic absorption spectrophotometer AA 6800. Osaka.

Selle, P., Ravindran, V., Partridge, G., 2009. Beneficial effects of xylanase and/or phytase inclusions on ileal amino acid digestibility, energy utilisation, mineral retention and growth performance in wheat-based broiler diets. Anim. Feed Sci. Technol. 153, 303–313. https://doi.org/10.1016/j.anifeedsci.2009.06.011.

Signor, A., Feiden, A., Boscolo, W., Signor, A., Gonçalves, G., Sary, C., Klein, S., 2013. Eventos reprodutivos do jundiá rhamdia voulezi cultivado em tanques-rede. Revista Brasileira de Reprodução Animal 37, 272–277.

Signor, A., Lewandowski, V., Silva, R.A.D., Fries, E.M., Schuller, J.M., 2016. Effect of phytase on digestibility of corn, sorghum and wheat bran by silver catfish (rhamdia voulezi). Acta Scientiarum. Animal Sciences 38, 355. https://doi.org/10.4025/actascianimsci.v38i4.32054.

da Silva, P.R.F., de Freitas, T.F.S., 2008. Biodiesel: o ônus e o bônus de produzir combustível. Ciência Rural 38, 843–851. https://doi.org/10.1590/s0103-84782008000300044.

Singh, P., 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: a review. World's Poultry Science Journal 64, 553–580. https://doi.org/10.1017/s0043933908000202.

Singh, B., Satyanarayana, T., 2014. Fungal phytases: characteristics and amelioration of nutritional quality and growth of non-ruminants. J. Anim. Physiol. Anim. Nutr. 99, 646–660. https://doi.org/10.1111/jpn.12236.

Sugiura, S.H., Gabaudan, J., Dong, F.M., Hardy, R.W., 2001. Dietary microbial phytase supplementation and the utilization of phosphorus, trace minerals and protein by rainbow trout [oncorhynchus mykiss (walbaum)] fed soybean meal-based diets. Aquac. Res. 32, 583–592. https://doi.org/10.1046/j.1365-2109.2001.00581.x.

Surek, D., Maiorka, A., Dahlke, F., Opalinski, M., Franco, S.G., Krabbe, E.L., 2008. Uso de fitase em dietas de diferentes granulometrias para frangos de corte na fase inicial. Ciência Rural 38, 1725–1729.

Suttle, N., 2010. Calcium. In: Mineral Nutrition of Livestock. CABI, pp. 54–91. https://doi.org/10.1079/9781845934729.0054.

Szkudelski, T., 1998. Phytic acid affects lipid metabolism in the rat. Journal of Animal and Feed Sciences *7*, 429–436. https://doi.org/10.22358/jafs/69317/1998.

Tudkaew, J., Gabaudan, J., Phromkunthong, W., 2008. The supplementation of phytase ronozyme p on the growth and the utilisation of phosphorus by sex-reversed red tilapia (oreochromis niloticus Linn.). Songklanakarin Journal of Science & Technology 30.

Vandenberg, G., Scott, S., la Noue, J.D., 2011. Factors affecting nutrient digestibility in rainbow trout (oncorhynchus mykiss) fed a plant protein-based diet supplemented with microbial phytase. Aquaculture Nutrition 18, 369–379. https://doi.org/10.1111/j.1365-2095.2011.00901.x.

Vielma, J., Lall, S.P., Koskela, J., Schöner, F.-J., Mattila, P., 1998. Effects of dietary phytase and cholecalciferol on phosphorus bioavailability in rainbow trout (oncorhynchus mykiss). Aquaculture 163, 309–323. https://doi.org/10.1016/s0044-8486(98)00240-3.

Wang, F., Yang, Y.-H., Han, Z.-Z., Dong, H.-W., Yang, C.-H., Zou, Z.-Y., 2008. Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (oncorhynchus mykiss walbaum). Aquac. Int. 17, 143–157. https://doi.org/10.1007/s10499-008-9187-5.

Yang, S.-D., Lin, T.-S., Liu, F.-G., Liou, C.-H., 2006. Influence of dietary phosphorus levels on growth, metabolic response and body composition of juvenile silver perch (bidyanus bidyanus). Aquaculture 253, 592–601. https://doi.org/10.1016/j.aquaculture.2005.09.002.

Yu, B., Chiou, P.W.S., 1997. The morphological changes of intestinal mucosa in growing rabbits. Laboratory Animals 31, 254–263. https://doi.org/10.1258/002367797780596301.

Zhang, C., Mai, K., Ai, Q., Zhang, W., Duan, Q., Tan, B., Ma, H., Xu, W., Liufu, Z., Wang, X., 2006. Dietary phosphorus requirement of juvenile japanese seabass, lateolabrax japonicus. Aquaculture 255, 201–209. https://doi.org/10.1016/j.aquaculture.2005.11.040.

Zhou, Q.-C., Yue, Y.-R., 2011. Apparent digestibility coefficients of selected feed ingredients for juvenile hybrid tilapia, oreochromis niloticus × oreochromis aureus. Aquac. Res. 43, 806–814. https://doi.org/10.1111/j.1365-2109.2011.02892.x.