

**Exigência de fósforo para juvenis de jundiá (*Rhamdia quelen*) em uma dieta semipurificada**

**Phosphorus requirement for juvenile jundiá (*Rhamdia quelen*) on a semi-purified diet**

**Requisito de fósforo para jundiá juvenil (*Rhamdia quelen*) en una dieta semipurificada**

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**Resumo**

O objetivo do presente estudo foi a determinação da exigência de fósforo total na dieta de alevinos de jundiá (*Rhamdia quelen*) em uma dieta semi-purificada. Realizou-se uma pesquisa experimental quantitativa, utilizando-se como fonte proteica a albumina desidratada, com a suplementação de fosfato de sódio monobásico em diferentes níveis: 0,04; 0,30; 0,47 e 0,70% de fósforo (P) total. Foram utilizados 160 peixes com peso médio de  $1,20 \pm 0,53$ g em um delineamento experimental o completamente casualizado, com quatro tratamentos e quatro repetições. O experimento teve duração de 33 dias. Para determinação das exigências de fósforo para os juvenis foram avaliados parâmetros composição química corporal, composição mineral óssea e de desempenho produtivo. A composição química corporal apenas os níveis de proteína não apresentaram diferença significativa ( $p>0,05$ ). Os níveis de fósforo, potássio e magnésio nos ossos aumentaram de forma linear, com aumento dos níveis de fósforo na dieta. Baseando-se no ganho de peso e comprimento total a exigência de fósforo para juvenis de jundiá (*Rhamdia quelen*) em uma dieta semipurificada está entre 0,53 - 0 58% de P-total.

**Palavras-chave:** Minerais; Crescimento; Alimento; Nutrição; Peixe.

### **Abstract**

The aim of the present study was to determine the total phosphorus requirement in the diet of jundiá fry (*Rhamdia quelen*) in a semi-purified diet. A quantitative experimental research was carried out, using dehydrated albumin as a protein source, with the supplementation of monobasic sodium phosphate at different levels: 0.04; 0.30; 0.47 and 0.70% of total phosphorus (P). 160 fish with an average weight of  $1.20 \pm 0.53$ g were used in an experimental or completely randomized design, with four treatments and four replications. The experiment lasted 33 days. To determine the phosphorus requirements for juveniles, body chemical composition, bone mineral composition and productive performance parameters were evaluated. The body chemical composition only the levels of protein did not present significant difference ( $p > 0.05$ ). The levels of phosphorus, potassium and magnesium in the bones increased linearly, with increased levels of phosphorus in the diet. Based on weight gain and total length, the requirement for phosphorus for juveniles of jundiá (*Rhamdia quelen*) in a semipurified diet is between 0.53 - 0.58% of P-total.

**Keywords:** Minerals; Growth; Food; Nutrition; Fish.

### **Resumen**

El objetivo del presente estudio fue determinar el requerimiento de fósforo total en la dieta de alevines (*Rhamdia quelen*) en una dieta semipurificada. Se realizó una investigación experimental cuantitativa, utilizando albúmina deshidratada como fuente de proteína, con la suplementación de fosfato de sodio monobásico a diferentes niveles: 0,04; 0,30; 0,47 y 0,70% del fósforo total (P). Se utilizaron 160 peces con un peso promedio de  $1,20 \pm 0.53$ g en un diseño experimental o completamente al azar, con cuatro tratamientos y cuatro repeticiones. El experimento duró 33 días. Para determinar los requisitos de fósforo para los juveniles, se evaluaron la composición química del cuerpo, la composición mineral ósea y los parámetros de rendimiento productivo. La composición química del cuerpo solo los niveles de proteína no presentaron diferencias significativas ( $p < 0,05$ ). Los niveles de fósforo, potasio y magnesio en los huesos aumentaron linealmente, con mayores niveles de fósforo en la dieta. Según el aumento de peso y la longitud total, el requerimiento de fósforo para los juveniles de jundiá (*Rhamdia quelen*) en una dieta semipurificada es de entre 0,53 y 0.58% del P-total.

**Palabras clave:** Mineral; Crecimiento; Comida; Nutrición; Pez.

## 1. Introduction

Aquaculture is an excellent option for the growing demand for proteins of animal origin. Currently, the production of food in this activity has been generating deep attention in society, leading to the development of research on the health quality of food, the production system used and the impact on the environment (Suárez-Mahecha et al., 2002).

Phosphorus and nitrogen are fundamental in fresh water eutrophication, being considered the main nutrients that pollute aquacultural environments (Bock et al., 2006).

The use of a balanced diet for the different cultivated species in addition to favoring growth reduces the levels of environmental pollutants and production costs. According to Fontagné et al. (2009) with the tendency to substitute fish meal for ingredients of vegetable origin, in fish feed the availability of phosphorus becomes highly variable and some deficiencies of this mineral may occur.

Due to the small number of nutritional studies, requirements of exotic species such as, for example, the channel catfish (*Ictalurus punctatus*) are often used to calculate the diet for jundiá, leading to excess or lack of nutrients in the rations.

The jundiá (*Rhamdia quelen*) is a species of the family Siluridae widely spread in natural and artificial rivers and ponds, in the southern part of South America it is well adapted to the cold of winter and has a rapid growth rate during the hottest months of the year (Soso et al., 2007).

Minerals can interact with each other, with other nutrients and also with some non-nutritive factors in the diet, and may be synergistic or antagonistic in the diet itself or during metabolism in the digestive tract (Moraes et al., 2009). The objective of the present research was to determine the total phosphorus requirements for juveniles of jundiá in a semipurified diet.

## 2. Material and Methods

A quantitative experimental research was carried out (Pereira, et al., 2018), using dehydrated albumin as a protein source, with the supplementation of monobasic sodium phosphate at different levels: 0.04; 0.30; 0.47 and 0.70% of total phosphorus (P). The experimental diets were formulated to determine the ideal levels of total phosphorus for juveniles of jundiá (*Rhamdia quelen*). The basal diet was formulated using albumin (1.72 g / kg of total phosphorus) as a protein source. To obtain the different levels of total phosphorus

in the diets (0.04; 0.30; 0.47 and 0.70%) sodium phosphate was included. Regarding calcium, a fixed level of 0.7% of the diet was maintained (NRC, 1993), with the addition of P.A. calcium carbonate (Table 1).

**Table 1.** Formulation and chemical composition of the diet.

	Experimental diets (phosphorus levels%)			
	0.04	0.30	0.47	0.70
<b>Ingredients (%)</b>				
Albumin <sup>1</sup>	48.00	48.00	48.00	48.00
Cellulose	23.80	22.60	22.00	20.20
Canola oil	4.00	4.00	4.00	4.00
Cod liver oil	4.00	4.00	4.00	4.00
Dextrin	13.00	13.00	13.00	13.00
Premix vitamin / micromineral <sup>2</sup>	1.00	1.00	1.00	1.00
Gelatine	2.00	2.00	2.00	2.00
Carboxymethylcellulose (CMC)	2.00	2.00	2.00	2.00
Calcium carbonate	1.20	1.20	1.20	1.20
Salt (NaCl)	1.00	1.00	1.00	1.00
Monobasic sodium phosphate	-	1.20	1.80	3.6
Total	100	100	100	100
<b>Chemical composition (%) (dry basis)</b>				
Crude protein	36.62	36.55	36.63	36.34
Crude lipid	8.81	8.75	9.17	9.23
Dry matter	93.81	93.42	93.55	93.23
Ash	5.47	6.48	6.66	7.81
Calcium	0.66	0.58	0.70	0.58
Digestible energy (kcal / kg) <sup>3</sup>	2.786	2.786	2.786	2.786
Total phosphorus	0.04	0.30	0.47	0.70

<sup>1</sup>Neonutri® brand albumin, with 80% crude protein and 0.17% total phosphorus

<sup>2</sup>Composition of vitamin and mineral supplement for fish: Manganese 15,000mg, Copper 3,000mg, Iron 25,000mg, Ac. Folico 1.500mcg, Zinc 30.000mg, Vit. B12 10,000mcg, Ac. Nicotinic 37,500mg, Vit. At 2,500UI / g, Vit. C 25,000mg, Ac. Pantothenic 20.000mg, Vit. D3 500UI / g, Vit. E 20,000mg, Biotin 50.00mcg, Vit. K33,500mg, Vit. B 17,000mg, Vit. B2 7.425mg, Vit. B6 7.250mg, Iodine 660mg, Selenium 110mg;

<sup>3</sup>Based on the digestible energy values proposed by NRC 1993 for fish (Super Crac Master 5.0® software).

<sup>4</sup>Based on the availability of albumin phosphorus (71%) and sodium phosphate (90%), for channel catfish (*Ictalurus punctatus*) Wilson et al. (1982). (Source: authors)

The ingredients were weighed and mixed gradually from the smallest to the largest volume. After these processes were mixed again in a blender, with the addition of warm water

(10%) to facilitate the homogenization of the ingredients. At the end of this process, the mixture was distributed in trays and dried in an oven at 50°C for 14 hours. These diets were packaged and stored in a freezer (-18°C) for later use. The chemical composition of the experimental diets was obtained through bromatological analysis (AOAC, 1995). For mineral analysis, the process of acid digestion and determination in atomic absorption spectrophotometry was performed (Tedesco et al., 1995).

Jundiá juveniles with an average weight of  $1.20 \pm 0.53$ g and  $5.38 \pm 0.26$  cm of total length were used. The fish were randomly distributed in 16 polypropylene boxes, with a useful volume of 200 liters. These experimental units had a biofilter system, artificial aeration with a central blower and an air-conditioned room for maintaining water temperature.

A completely randomized design with four treatments and four replications was used. The fish were acclimated with a commercial diet for 10 days, for later introduction of experimental diets. The experimental period was 33 days with a feed rate of 10% of the biomass, in four portions a day (8:00 am, 10:00 am, 2:00 pm and 5:00 pm). At the end of the experiment, the animals were biometrically weighed and measured.

The water quality was monitored three times a week, controlling the dissolved oxygen levels and temperature (digital oximeter model 55 from YSI), pH (potentiometer model AT 310), total phosphorus (photocolorimeter AT-100PB), total ammonia, following methodology suggested by APHA (1998).

The productive performance of the animals was evaluated through the final weight, PF (g); average weight gain, [GP (g) = final-initial weight]; total length, CT (cm); specific growth rate, TBI (%) =  $[(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{days of experiment}] \times 100$ ; apparent feed conversion, CAA =  $(\text{food consumed} / \text{weight gain}) \times 100$  and survival S (%) =  $[\text{survivors} / \text{initial population}] \times 100$ .

At the end of the experimental period, fish were euthanized by deepening anesthetic (200 mg/L benzocaine solution) with subsequent storage in a freezer at -18°C for future evaluation of body and mineral chemical composition.

To obtain the bones of the spine, the fish carcasses were kept in heated water (80°C) for approximately two minutes and, immediately afterwards, dissected with the aid of forceps (Furuya et al., 2001).

These samples were digested in acidic solution (Silva et al., 2004) and the concentrations of P, Ca, K and Mg in triplicate were determined at the Chemical Analysis Laboratory of the Soil Department of the Faculty of Agronomy Eliseu Maciel of the Federal University of Pelotas, through atomic absorption spectrophotometry (Tedesco et al., 1995).

The other fish were subjected to pre-drying with forced air circulation at a temperature of 50 °C for 72 hours, proceeding to grinding, conditioning and freezer storage for further analysis of body chemical composition.

The methodology described by the Association of Official Analytical Chemists (1995) was used where: humidity was obtained by drying in an oven at 105 °C until constant weight. After this process, aliquots of these samples were used for the analysis of crude lipid (Sohxlet extractor), crude protein (N x 6.25), by the Microkjeldahl method, and ash (muffle at 550 °C for 6 hours).

The data were submitted to analysis of variance (ANOVA), when a difference was found between the means ( $P \leq 0.05$ ), regression analysis was performed (STATÍSTICA®, 1995).

### 3. Results and Discussions

The physical-chemical parameters of the water remained within the appropriate limits for the species (Piedras et al., 2004), showing no difference between treatments. The average results were: temperature of  $23.51 \pm 2.3^\circ\text{C}$ , dissolved oxygen  $5.08 \pm 0.65$  mg/L, pH  $7.8 \pm 0.70$ , ammonia  $0.02 \pm 0.0$  mg/L, phosphorus total  $0.30 \pm 0.13$  mg/L and alkalinity  $45 \pm 5$  mg/L.

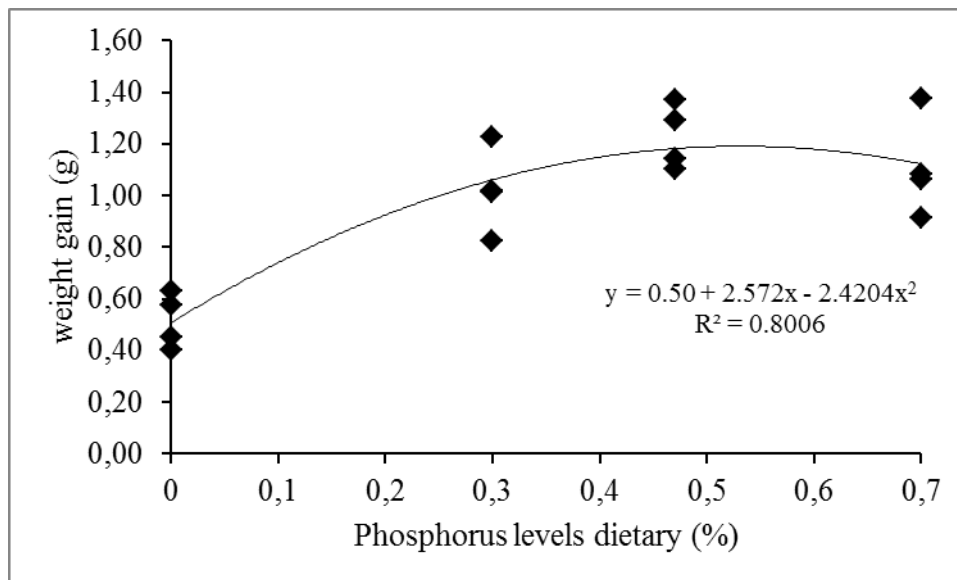
The survival of juvenile jundiá was not affected by the levels of phosphorus in the diet, with  $97.5 \pm 5\%$  for the control diet (0.04%),  $95 \pm 5.77\%$  for the level of 0.30% of phosphorus in the diet,  $92.5 \pm 9.57\%$  for a diet with 0.47% phosphorus and  $97.5 \pm 5\%$  for a diet with 0.70% total phosphorus ( $p > 0.05$ ).

The average final weight of jundiá juveniles showed a quadratic effect with increased levels of total phosphorus in the diet, with an estimated phosphorus requirement of 0.53% in the diet for greater final weight, according to the polynomial equation ( $1.70 + 2.52x - 2.35x^2$ ,  $R^2 = 0.81$ ).

The polynomial adjustment shows that 0.53% of total phosphorus results in greater weight gain and 0.58% in greater total length (Figures 1 and 2). In a study by Bueno et al. (2012) the addition of 0.4% of phosphorus in the diet of juvenile Nile tilapia (*Oreochromis niloticus*) led to the addition of 55% of phosphorus in the water, this highlights the importance of using efficient diets to reduce pollution environmental. For fingerlings of Indian carp (*Labeo rohita*) 0.65% phosphorus in the diet was recommended for greater growth (Musharraf and Khan, 2018).

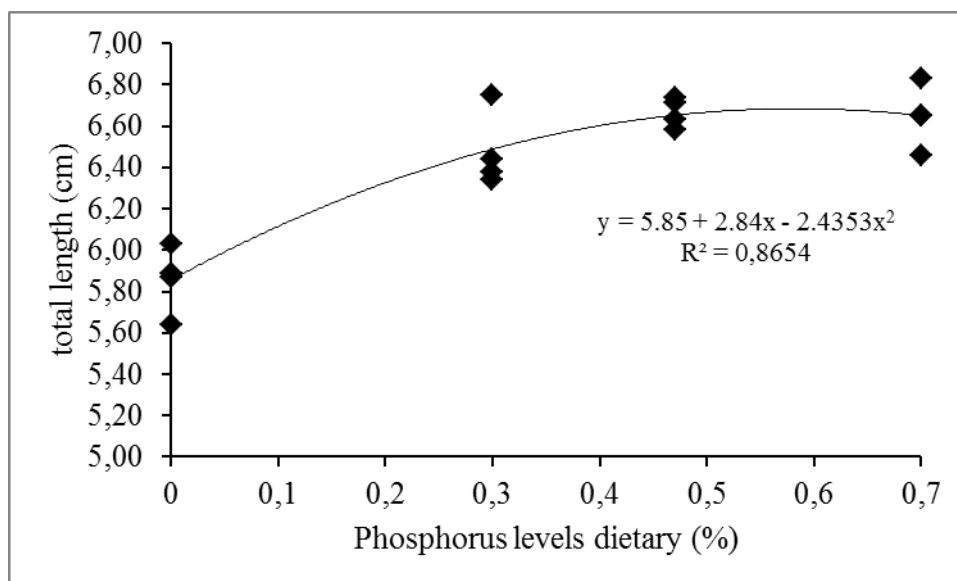
Nwanna et al. (2009) report that levels of 6.7 to 8.2 g / kg are efficient for good performance for the African catfish (*Clarias gariepinus*).

**Figure 1.** Weight gain of jundiá juveniles fed different levels of phosphorus in a semi-purified diet.



Source: Authors.

**Figure 2.** Total length of juvenile jundiá fed different levels of phosphorus in a semi-purified diet.



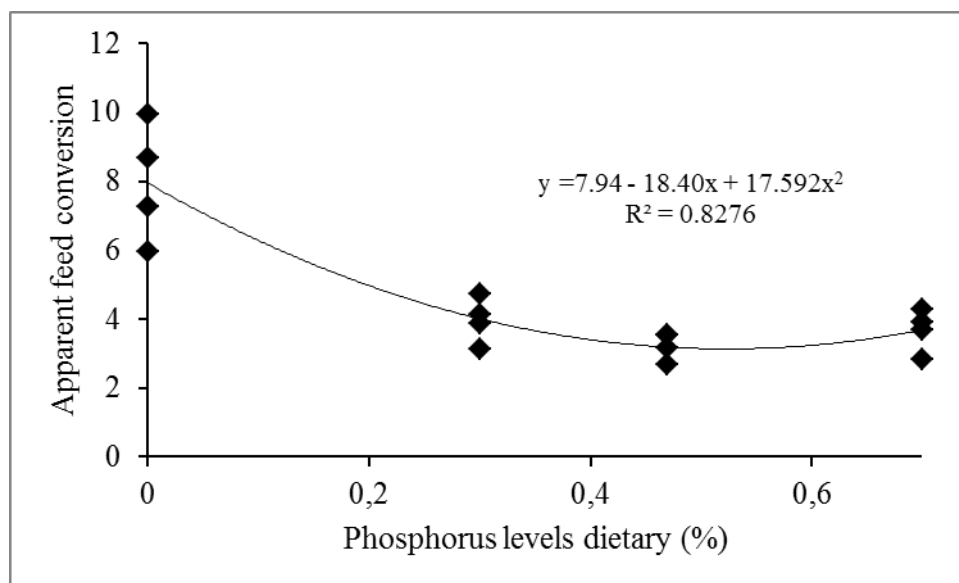
Source: Authors.

Levels of 0.62% of available phosphorus were sufficient for maximum growth for European white fish (*Coregonus lavaretus*) (Vielma et al., 2002). According to the polynomial equation ( $y = 1.06 + 3.01x - 3.72x^2$ ,  $R^2 = 0.79$ ) 0.40% of total phosphorus was sufficient for a higher specific growth rate.

One factor to be considered for assessing phosphorus requirements in the fish diet is the existence of antinutritional factors such as the phytate present in oilseeds (soybean meal, corn, etc.). According to NRC (1993), the diets used to assess nutritional requirements must be highly chemically defined, with gelatin and casein being a good protein combination.

The apparent feed conversion reduced in a quadratic way with the increase in the levels of phosphorus in the diet, with 0.52% of phosphorus in the diet providing better results (Figure 3). Zafar and Khan (2018) also observed a better feed conversion with increased levels of phosphorus in the diet of stingray catfish (*Heteropneustes fossilis*).

**Figure 3.** Apparent feed conversion of juvenile jundiá fed different levels of phosphorus in a semi-purified diet.



Source: Authors.

The crude body protein of jundiá juveniles did not show a significant effect with an increase in dietary P levels ( $p > 0.05$ ) (Table 1), the same was observed by Araújo et al. (2016) for tambaqui juveniles (*Colossoma macropomum*).



**Table 1.** Centesimal composition (g per 100 g) of carcasses of juvenile jundiá (*Rhamdia quelen*) fed with increasing levels of phosphorus in the diet.

Treatments	Body composition (%)			
	Crude protein	Crude Lipid	Ash	Dry matter
0.04	61.19±0.59	16.09±2.19	12.65±0.89	22.80±0.36
0.30	63.19±2.96	17.36±0.26	13.19±1.05	23.04±0.62
0.47	62.11±2.54	15.09±0.38	13.88±0.97	23.90±0.36
0.70	62.69±0.74	14.24±0.44	15.98±0.02	22.71±0.26
p	0.664	0.048 <sup>1</sup>	0.001 <sup>2</sup>	0.033 <sup>3</sup>

<sup>1</sup>Linear decreasing effect  $y = 16.78 - 2.80x$  ( $R^2 = 0.24$ ), <sup>2</sup>Increasing linear effect  $y = 12.24 + 4.59x$  ( $R^2 = 0.66$ ), <sup>3</sup>Square effect  $y = 22.72 + 4.20x - 5.73x^2$  ( $R^2 = 0.32$ ) Source: Authors.

However, the crude lipid reduced linearly and body ash also increased linearly with an increase in phosphorus levels in the diet. Similar results were found by Uyan et al. (2007) where the increase in phosphorus levels in the diet reduced body fat in juveniles of Japanese sole (*Paralichthys olivaceus*).

In a study by Pezzato et al. (2006) the body crude lipid of Nile tilapia fry reduced with increasing levels of phosphorus in the diet in a quadratic way Zhang et al. (2006) and Xie et al. (2016) also observed an increase in body ash from juveniles of Japanese seabass (*Lateolabrax japonicus*) and gibel carp (*Carassius auratus gibelio* var. CAS III), respectively. Coloso et al. (2003), observed no effect of phosphorus levels on body ash in the rainbow trout (*Oncorhynchus mykiss*) diet.

The increase in phosphorus levels in the diet quadratically affected the body dry matter of juveniles. For catfish (*Ictalurus punctatus*) the increase in phosphorus levels in the diet increased dry matter and muscle protein (Eya & Lovell, 1997).

The calcium composition of the jundiá juvenile vertebrae was not affected by the levels of phosphorus in the diet ( $p > 0.05$ ) (Table 2).

**Table 2.** Mineral levels in the bones of juvenile jundiá (*Rhamdia quelen*) fed with increasing levels of total phosphorus in the diet.

Minerals in bones (g/kg)	Total phosphorus levels in the diet (%)					Regression
	0.04	0.3	0.47	0.70	p	
Ca	89.59±3.75	88.97±3.39	93.66±0.71	94.60±3.29	0.124	-
P	48.41±2.41	48.18±0.90	54.29±2.58	58.05±0.34	0.001	y=14.67x+46.84.R <sup>2</sup> =0.71
K	8.54±0.13	9.04±0.15	8.32±0.15	9.13±0.31	0.002	y=0.53x+8.56. R <sup>2</sup> =0.13
Mg	3.03±0.21	2.90±0.11	3.13±0.07	4.01±0.31	0.002	y=1.32x + 2.78. R <sup>2</sup> =0.52

Ca (calcium), P (phosphorus), K (potassium) and Mg (magnesium). Source: Authors.

What differs from the data obtained by Rocha et al. (2014) and Liang et al. (2011) where the levels of phosphorus in the diet influenced the calcium deposition in the vertebrae of pejerrey fingerlings (*Odontesthes bonariensis*) and juvenile grass carp (*Ctenopharyngodon idella*), respectively. However, the levels of P, K and Mg had an increasing linear effect with increasing levels of P in the diet.

Pejerrey fingerlings (*Odontesthes bonariensis*), feed with semi-purified diets with phosphorus levels between 0.09% to 0.83% provided a quadratic effect on the deposition of P in the bones (Rocha et al. 2014), which differs from what was observed in this experiment, possibly the inclusion of a higher level of phosphorus in the diet could reveal the plateau of the deposition of the mineral.

The levels of phosphorus in the bones of the vertebrae increased up to the level of 0.9% of available phosphorus in the diet for juveniles of Japanese seabass (*Lateolabrax japonicus*), for the yellow catfish (*Pelteobagrus fulvidraco*) 0.78% (Luo et al. 2010 ).

Magnesium and potassium levels increased linearly with increasing levels of phosphorus in the diet. Unlike what was observed by Roy et al. (2003), who found reduced levels of magnesium in the bones of the vertebrae of juvenile haddock (*Melanogrammus aeglefinus* L.) with an increase from 0.42% to 0.82% of phosphorus in the diet, and no influence on potassium. According to this author, a reduction in the deposition of other minerals in the bones may be due to the competitive inhibition of these cations during intestinal absorption.

#### 4. Conclusions and Suggestions

The inclusion of increasing levels of phosphorus in the diet linearly increases the mineral deposition of P, K and Mg in the bones, however, studies with higher levels of phosphorus in the diet should be carried out to determine the mineral deposition plateau in the bones.

Based on production performance data, the phosphorus requirement for juveniles of jundiá in a semipurified diet is between 0.53 – 0.58% of P-total

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