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Research Article

Dietary total phosphorus supplementation in goldfish diets

Weslley F. Braga[1,2], Janaína G. Araújo[1], Graciela P. Martins[1]
Silvio L. Oliveira[1] & Igo G. Guimarães[1]
[1]Laboratório de Pesquisa em Aquicultura, Universidade Federal de Goiás
Campus Jataí, Jataí, GO, C.P. 37580-615, Brazil
[2]Universidade Federal de Lavras, Lavras, MG, C.P. 37200-000, Brazil
Corresponding author: Igo G. Guimarães (igoguimaraes@ufg.br)

ABSTRACT. Although goldfish (*Carassius auratus*) is an important species for the ornamental fish industry, few data are available regarding the nutrient requirement of this species, with emphasis to mineral nutrition. Thus, we designed a 45-day feeding trial to evaluate the effect of dietary total phosphorus (P) levels on growth performance and carcass mineral composition of goldfish fingerlings. 210 goldfish with 1.18 ± 0.04 g were randomly stocked into 30 3L-aquaria in a completely randomized design. Test diets were formulated to contain the following dietary total P levels: 3.5, 6.5, 9.5, 12.5 and 15.5 g kg^{-1}. Dietary P affected all growth parameters and carcass macrominerals deposition, however, the micromineral carcass composition was not affected. No P deficiency signs were observed throughout the experiment. The linear broken-line model best fitted to daily weight gain, feed conversion ratio, specific growth rate, protein efficiency ratio, P retention, and whole-body P concentration at 8.2, 11.4, 8.2, 11.4, 15.5 and 7.1 g kg^{-1} dietary P, respectively. An exponential model best fitted to phosphorus utilization data with an estimated requirement of 8.6 g kg^{-1}. In sum, the use of total P levels between 7.13 and 11.4 g kg^{-1} in goldfish diets seems to meet the requirement for maximum growth, feed utilization and proper whole-body mineralization.

Keywords: *Carassius auratus*, phosphorus requirement, minerals, nutrition, ornamental fish, aquaculture.

Suplemento de fósforo total en dietas para carpa dorada

RESUMEN. Aunque la carpa dorada *Carassius auratus* es una especie importante para la industria de peces ornamentales, existen escasos estudios sobre las necesidades nutricionales de esta especie, con énfasis en la nutrición mineral. Por lo tanto, se diseñó un estudio de alimentación de 45 días para evaluar el efecto de los niveles dietarios de fósforo (P) total sobre el crecimiento y la composición mineral del esqueleto de los alevines de *C. auratus*. 210 peces con peso inicial promedio de 1,18 ± 0,04 g se colocaron aleatoriamente en 30 acuarios de 3 L en un diseño completamente al azar. Las dietas se formularon para contener los siguientes niveles de fósforo dietario total: 3,5; 6,5; 9,5; 12,5 y 15,5 g kg^{-1}. El fósforo dietario afectó todos los parámetros de crecimiento y la deposición de minerales en el esqueleto; sin embargo, la composición del elemento traza en la carcasa no fue afectada. No se observaron signos de deficiencia de fósforo a lo largo del experimento. El modelo lineal de línea quebrada mostró el mejor ajuste para la ganancia diaria en peso, conversión alimenticia, tasa de crecimiento específico, tasa de eficiencia proteica, retención de P y concentración de P en todo el cuerpo con el siguiente requerimiento estimado: 8,2; 11,4; 8,2; 11,4; 15,5 y 7,1 g kg^{-1}, respectivamente. El modelo exponencial presentó el mejor ajuste a los datos de utilización de fósforo, con un requerimiento estimando de 8,6 g kg^{-1}. El uso de los niveles de fósforo entre 7,13 y 11,4 g kg^{-1} en las dietas de *C. auratus* cumple con los requisitos para el crecimiento máximo, utilización de alimento y adecuada mineralización del cuerpo.

Palabras clave: *Carassius auratus*, requerimiento de fósforo, minerales, nutrición, peces ornamentales, acuicultura.

INTRODUCTION

Ornamental fish industry is an aquaculture sector which has been growing in the last decades and has become a main economic alternative in some developing countries. Marine and freshwater species have been used as pets and are kept in homes under several types of environments using different shapes and sizes of

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aquaria. Among the ornamental fish species, goldfish is one of the top five most produced freshwater fishes worldwide. This species has been used not only as a pet, but as a model in several fish physiology studies and selective breeding because of its long-time domestication and easy handling (Rosa *et al*., 1994; FAO, 2007).

Despite the importance of goldfish for the ornamental fish industry, few nutrient requirement data are available. Until the present time, there are reports mainly concerning on protein and amino acids requirement (Lochmann & Phillips, 1994; Fiogbé & Kestemont, 1995; Snellgrove & Alexander, 2011). However, the micronutrients requirement data are scant for the goldfish, mainly in respect to mineral nutrition.

Although the importance of phosphorus nutrition is well known among fish nutritionists, mainly due to its effect on bone development and kinetics of energy transfer in the cell (Lall, 2002; Uyan *et al*., 2007; NRC, 2011), few studies are available on the quantitative phosphorus requirement for ornamental fish, including goldfish. Besides the importance of phosphorus nutrition for the adequate growth of fish and bone mineralization, it is well established that the excess phosphorus in fish diets may promote eutrophication of water bodies and thus, reducing the sustainability of aquaculture production (Lall, 2002). Quantitative phosphorus requirement for fish is between 2.5 and 10 g kg^{-1} diet. The requirement may be variable according to the life stage, phosphorus source and the statistical approach used to estimate the requirement (Pezzato *et al*., 2006; NRC, 2011). Additionally, there are strong evidences that digestive tract differences among fishes may influence the quantitative requirement of phosphorus (Hua & Bureau, 2006, 2010). Thus, studies on phosphorus nutrition for ornamental fish is of utmost importance once when diets are formulated with adequate phosphorus levels and sources, reduced phosphorus loading to the environment is expected, improving the welfare and lifespan of the fish. Therefore, it is important to develop proper formulated diets for this species, considering that the consumers may be more prone to pay higher prices for a diet which reduces the algae bloom in aquaria and increases the life span of the fish. Based on the lack of information on mineral nutrition, with emphasis to phosphorus, and its importance for the growth and health maintenance of the fish, we designed an experiment to determine the effects of phosphorus supplementation on growth performance and carcass mineral composition of goldfish fingerlings. Additionally, we provide an estimate of the total P requirement for this species.

MATERIALS AND METHODS

All experimental procedures were approved by the Animal Ethics Committee of the Universidade Federal de Goiás (protocol 301/10-CEUA).

Experimental procedure

Five hundred goldfish with 90 days-old spawned from our wild variety of goldfish broodstocks were selected and acclimatized to the laboratory conditions in two 500 L-aquaria. These fish were fed twice daily with a commercial diet (280 g kg^{-1}) to satiation for two weeks. The feeding trial was conducted in a recirculating system; accumulated feces were removed by siphon. A homogenous group of 210 goldfish was selected by weight (1.18 ± 0.04 g) and randomly stocked into 30 3L-aquaria.

Each diet was fed to six groups of fish for 45 days. Fish were fed until apparent satiation at 07:00, 12:00, and 17:00 h. During the feeding trial water quality parameters were maintained in the optimum range for fish rearing (pH 6.8 ± 0.3, dissolved oxygen 5.8 ± 0.7 mg L^{-1} and ammonia (NH_3) 124 μg L^{-1}). Water temperature was heater-controlled and kept at 26 ± 0.7ºC. All aquaria were maintained under natural photoperiod. Dissolved P levels in the water varied from 0.34 to 0.63 mg L^{-1}.

During the experiment, fish mortality was recorded. At the beginning and at the end of the feeding experiment, fish were starved for 24 h, and then weighed by group.

Experimental diets

Diets were manufactured using conventional feed ingredients to contain the same digestible energy (12.54 MJ DE kg^{-1} diet) and protein content (280 g kg^{-1}) according to previous studies conducted in our laboratory (Souto *et al*., 2013). An unsupplemented diet with no adding dicalcium phosphate was formulated as the control diet and by adding dicalcium phosphate the following dietary total P level were obtained: 6.5; 9.5; 12.5 and 15.5 g kg^{-1} (Table 1). The unsupplemented diet contained 3.5 g kg^{-1} total P.

All ingredients were grounded until sieve in a mesh diameter of 500 μm. Diets were mechanically mixed with water (25% dry weight) and the moist mixture was extruded in a 4 mm die of a meat grinder. Diets were oven dried until present moisture <100 g kg^{-1}, and stored at -18ºC until further use. At the beginning of the experiment diets were ground and sieved in a mesh diameter according to fish size.

Table 1. Experimental diets composition and proximate analysis.

Ingredient	Total phosphorus levels (g kg^{-1})				
	3.5	6.5	9.5	12.5	15.5
Soybean meal	517.0	517.0	517.0	519.0	522.0
Yeast	20.0	20.0	20.0	20.0	20.0
Cottonseed meal	20.0	20.0	20.0	20.0	22.0
Corn	276.2	272.2	266.0	255.0	233.0
Broken rice	90.0	90.0	90.0	90.0	90.0
DL-Methionine	2.0	2.0	2.0	2.0	2.0
Soybean oil	30.0	30.0	31.0	34.4	39.4
Dicalcium phosphate	0.0	16.0	32.5	48.5	65.0
Limestone	38.2	26.2	14.9	4.5	0.0
Vitamin C	0.4	0.4	0.4	0.4	0.4
NaCl	1.0	1.0	1.0	1.0	1.0
Vitam/min mix[1]	5.0	5.0	5.0	5.0	5.0
BHT[2]	0.2	0.2	0.2	0.2	0.2
Total	1000	1000	1000	1000	1000
Proximate composition (g kg^{-1})					
Digestible energy[3] (MJ kg^{-1})	12.62	12.57	12.54	12.57	12.56
Digestible protein[3]	251.9	251.6	251.3	251.4	252.0
Crude protein[4]	280.9	280.6	280.1	280.1	280.2
Crude fiber[4]	48.3	48.2	48.1	48.0	48.3
Starch[4]	307.6	305.1	301.2	294.6	281.3
Lipid[4]	60.3	60.1	60.9	63.8	67.9
Calcium[4]	20.9	20.3	20.0	20.0	22.3
Phosphorus[4]	4.4	6.9	10.9	13.6	18.2

[1]Vitamin and mineral mix provided the following (mg or IU kg^{-1} of mixture): folic acid 600 mg, biotin 24 mg, choline chloride 54 g, niacin 12000 mg, calcium pantothenate 6000 mg, vitamin A 600000 UI, vitamin B1 2400 mg, vitamin B12 2400, mg, vitamin B2 2400 mg, vitamin B6 2400 mg, vitamin C 24 g, vitamin D3100000 UI, vitamin E 6000 mg, vitamin K3 1200 mg. Co 1 mg Cu 300 mg, Fe 5000 mg, I 10 mg, Mg 2000 mg, Se 10 mg, Zn 3000 mg. [2]Antioxidant: butylated hydroxytoluene. [3]Calculated according to reported values for carp and tilapia (Pezzato *et al.*, 2006; NRC, 2011), [4]Analysed values.

Proximal and mineral analysis

A group of 20 fish at the beginning of the experiment and four fish per aquaria at the end were collected and euthanized with high benzocaine concentration (193 mg L^{-1}). Fish were ground in a meat mincer and samples stored frozen (-18°C) to determine the whole-body chemical composition and mineral content. Proximate composition analysis and mineral content of feed ingredients, experimental diets and whole fish were performed by the standard methods of AOAC (1995). Samples of diets were dried to a constant weight at 105°C to determine moisture. Protein was determined by measuring nitrogen (N x 6.25) using the Kjeldahl method, lipid by ether extraction using Soxhlet, ash by combustion at 550°C, crude fiber by fritted glass crucible method after treated with H_2SO_4 and NaOH. Samples of fish whole-body were analyzed to determine calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe) and zinc (Zn) concentrations by flame atomic absorption spectrophotometry on a Shimadzu AA-6800 (Shimadzu, Japan), while total P concentration was analyzed by a colorimetric process, using the vanado-molybdate reagent.

Data analysis

The following variables were calculated: feed intake FI = feed consumption (g)/((FW +IW)/2)×t); daily weight gain DWG = (FW−IW)/t; feed conversion ratio FCR = dry feed fed in g/wet weight gain in g; specific growth rate SGR = (ln FW−ln IW)×100/t ; protein efficiency ratio PER = wet weight gain in g/protein intake in g; phosphorus utilization PU = weight gain in g/phosphorus intake, g; protein productive value PPV = (FW × CP1−IW × CP2)/(Id × CP); phosphorus retention PR = (FW × P1−IW × P2)/(Id × P).

Where FW is final body weight, IW is initial body weight, t is experimental duration in days, Id is feed intake on a dry matter basis. CP, CP1 and CP2 represent protein contents in diet; final fish body and initial fish body, respectively, P, P1 and P2 represent phosphorus content in diet, final fish body and initial fish body, respectively.

Statistical analysis

The experiment followed a completely randomized design with five treatments and six replicates. The data were verified for normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's *F* test). The concentration of dietary total P was the fixed factor in this study. Data were analyzed using PROC GLM (SAS Institute, Inc., Cary, NC, USA) for a one-way analysis of variance (ANOVA) and GraphPad Prism 6.02 (Graphpad software, San Diego, CA) was used for graphs preparation. When the quadratic term in the model was statistically significant, the relationship between analyzed phosphorus level and the measured parameter was further evaluated using PROC NLIN to determine the response curve and estimate the phosphorus requirement in relation to the measured parameter. The models used to estimate the requirement were selected based on the least sum of squared differences between the values of the observed and predicted values of the dependent variable (Shearer, 2000). For response variables that were significantly different and no model was able to be fitted, pairwise comparisons between treatments means were made using the Student Newman Keuls Multiple Range Test. A significance level of $P < 0.05$ was used for all statistical analyses.

RESULTS

After 45 days of feeding the experimental diets, all growth parameters were significantly affected by the phosphorus levels, except for the feed intake (Table 2). Analyzed total phosphorus levels were 4.4; 6.9; 10.9; 13.6 and 18.2 g kg^{-1} for diets formulated to contain 3.5; 6.5; 9.5; 12.5 and 15.5 g kg^{-1}, respectively. No signs of phosphorus deficiency were observed in this study throughout the experimental period.

The linear broken-line model best fitted to the DWG, FCR and SGR (Fig. 1). The estimated requirement for these parameters was 8.2, 11.4 and 8.2 g kg^{-1} total P, respectively. Feed intake was not affected by the dietary levels of total phosphorus in goldfish diets ($P > 0.05$).

Except for the PPV ($P < 0.05$), the dietary levels of phosphorus significantly affected the utilization of phosphorus and protein in goldfish (Table 3). The estimated requirement based on the linear broken-line model was 11.4, 15.5 and 7.1 for PER (Fig. 2a), PR (Fig. 2c) and whole-body phosphorus content (Fig. 2c), respectively. An exponential model best fitted to the phosphorus utilization (PUR) data and the estimated requirement (95% of the plateau) was 8.6 g kg^{-1} (Fig. 2b).

P supplementation significantly affected the macro mineral composition of goldfish carcass ($P < 0.05$). However, this effect was not evident for Mn, Fe and Zn (Table 4) ($P > 0.05$). Goldfish fed the unsupplemented diet showed the lowest whole-body calcium (Ca) content while fish fed diets with the highest P level (18.2 g kg^{-1}) had the highest Ca concentration. The broken-line model best fitted to the whole-body P content with an estimated requirement of 7.1 g kg^{-1} total P (Fig. 2d).

DISCUSSION

In this study, a slightly higher P requirement is reported for goldfish (8.2 g kg^{-1}) compared to the other species of the same group. Total P requirement for culture cyprinid fishes, specifically for common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*), has been reported to range from 6.0 to 8.49 g kg^{-1} (Nwanna *et al*., 2010; NRC, 2011; Liang *et al*., 2012), which is very close to the values observed in our study. The same P requirement observed in this study for both DWG and SGR indicates the close relationship of these growth parameters for goldfish. These small differences on P requirement among studies can be attributed to the rearing system used in the experimental procedure, P availability in different sources and ingredients and statistical model used to estimate the requirement.

No effect of total P levels on feed intake of goldfish was observed in this study. Although some studies with fish have reported similar results (Oliva-Teles & Pimentel-Rodrigues, 2004; Ribeiro *et al*., 2006; Furuya *et al*., 2008; Nwanna *et al*., 2010), others have reported a significant effect of phosphorus on this parameter (Yang *et al*., 2006; Furuya *et al*., 2008). These differences among studies may be related to different ingredient composition of the experimental diets.

The estimated requirement based on FCR (11.4 g kg^{-1}) was 39% higher than the requirement estimated for growth. This supports the results of previous studies with other fish species which observed a significant improvement on feed utilization when diets were supplemented with P in the form of dicalcium phosphate (Dato-Cajegas & Yakupitiyage, 1996; Pezzato *et al*., 2006). However, there are no reports showing any effect on feed utilization among P-supplemented diets (Pimentel-Rodrigues & Oliva-Teles, 2001; Luo *et al*., 2009; Nwanna *et al*., 2010). Differences among studies are most likely to occur when the experimental diets are formulated with plant protein ingredients since fish species may digest these products differently (Gatlin *et al*., 2007; Hardy, 2010). Furuya *et al*. (2004) reported that Nile tilapia improved

Table 2. Growth performance of goldfish fingerlings fed diets containing different levels of total phosphorus (n = 6, mean ± SD). Feed intake (FI), daily weight gain (DWG), feed conversion ratio (FCR), specific growth rate (SGR).

P levels (g kg^{-1})	FI (g)	DWG (mg day^{-1})	FCR	SGR (% day^{-1})
3.50	7.96 ± 0.85	3.81 ± 0.90	6.86 ± 1.28	0.46 ± 0.10
6.50	8.13 ± 0.31	4.51 ±0.27	5.75 ± 0.50	0.53 ± 0.04
9.50	8.70 ± 0.60	5.52 ± 0.73	5.07 ± 0.76	0.64 ± 0.08
12.50	8.56 ± 0.51	6.16 ± 0.73	4.47 ± 0.68	0.72 ± 0.08
15.50	7.84 ± 0.55	5.27 ± 0.36	4.73 ± 0.28	0.61 ± 0.05
ANOVA *P*-value	0.1315	0.0421	0.0007	0.0002
Regression				
Quadratic *P*-value	0.0592	0.0362	<0.0001	0.0001
Broken-line *P*-value	0.1051	0.0429	0.0015	0.0156

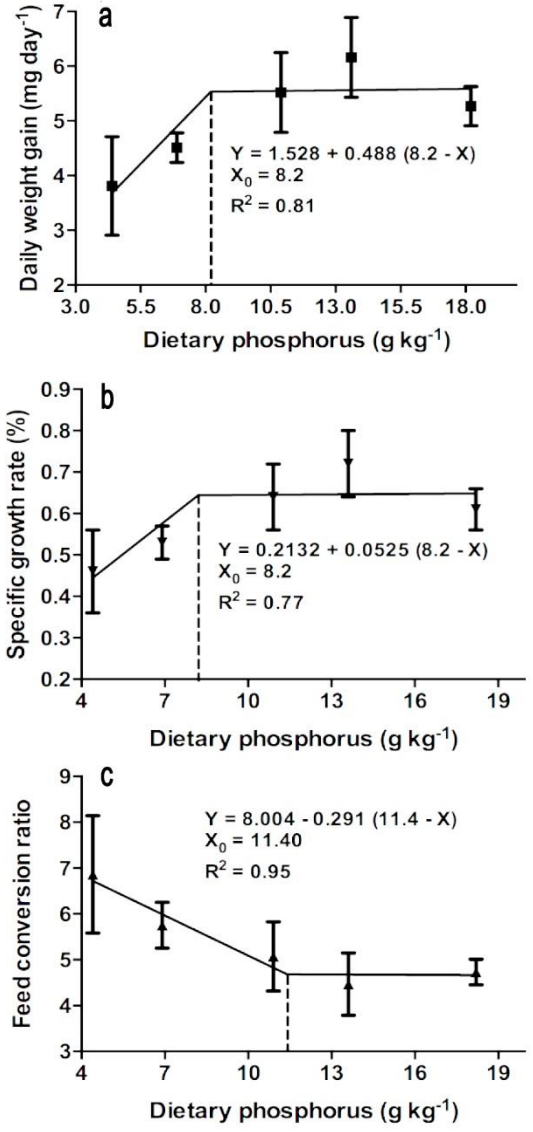

Figure 1. Daily weight gain (a), specific growth rate (b), and feed conversion ratio (c) of goldfish fingerlings fed diets containing graded levels of phosphorus. Each point is a mean of 6 aquaria ± SD.

FCR comparable to fish meal-based diets when not only limiting amino acids but also phosphorus was supplemented to plant-based diets. Thus, the higher requirement for FCR observed for goldfish in this study may be related to the plant-based diet used.

The lowest growth performance and feed utilization of goldfish fed the P unsupplemented diet (4.4 g kg^{-1} total P) may be a result of P deficiency, causing a slightly reduction in feed intake and feed utilization, and leading to a reduced energy supply to support growth of fish, and therefore, reducing the growth performance. Similar results were reported for Nile tilapia when fish were fed P-deficient diets (Watanabe *et al*., 1980; Dato-Cajegas & Yakupitiyage, 1996; Miranda *et al*., 2000).

P supplementation significantly affected the protein utilization of goldfish (Fig. 1a, Table 3) and the dietary level of 11.4 g kg^{-1} was sufficient to prevent this deficiency symptom. The reduced protein utilization in goldfish fed the unsupplemented P diet may be attributable to the impaired fatty acid utilization in the β-oxidation, once this mineral is an important co-factor for activation of some regulatory enzymes in this metabolic pathway. Therefore, leading to the use of protein as an energy source to support the basal metabolism (Roy & Lall, 2003). Earlier studies have reported that low levels of P in carp diets may affect the amino acid utilization for protein synthesis diverting their use for energy supply via gluconeogenesis (Onishi *et al*., 1981). However, the effect of P on nitrogen utilization of fish seems to be extremely variable among studies. For instance, European sea bass (*Dicentrarchus labrax*) (Oliva-Teles & Pimentel-Rodrigues, 2004) and gilthead sea bream (*Sparus aurata*) (Pimentel-Rodrigues & Oliva-Teles, 2001) seems to have the same P requirement for maximum growth (5.7 and 6.5 g kg^{-1}, respectively) and improved nitrogen utilization, however, reduced protein utilization

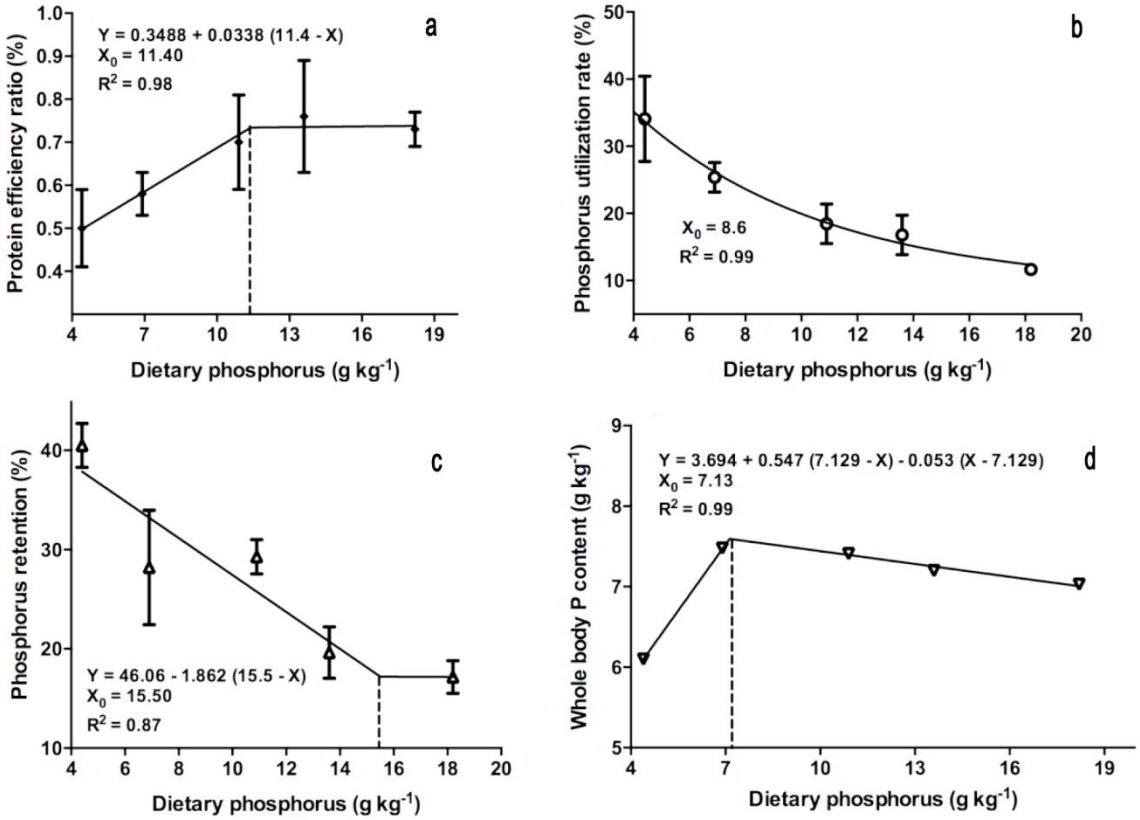

Figure 2. Protein efficiency ratio (a), phosphorus utilization rate (b), phosphorus retention (c) and whole-body P content (d) of goldfish fingerlings fed diets containing graded levels of phosphorus. Each point is a mean of 6 aquaria ± SD.

Table 3. Protein and phosphorus utilization of goldfish fed diets containing different levels of total phosphorus (n = 6, mean ± SD). Means followed by the different letters in the column are different at $P < 0.05$ by SNK test. Protein efficiency ratio (PER), phosphorus utilization rate (PUR), protein productive value (PPV), phosphorus retention (PR), *the exponential model best fitted to PUR data with a *P*-value of 0.0015.

P levels (g kg $^{-1}$)	PER (%)	PUR (%)	PPV (%)	PR (%)
3.50[1]	0.50 ± 0.09	34.08 ± 6.35	9.93 ± 1.70^{c}	40.49 ± 2.21
6.50	0.58 ± 0.05	25.37 ± 2.21	14.32 ± 0.43^{a}	28.20 ± 5.78
9.50	0.70 ± 0.11	18.44 ± 2.95	14.44 ± 1.18^{a}	29.28 ± 1.74
12.50	0.76 ± 0.13	16.79 ± 2.93	11.43 ± 1.12^{b}	19.62 ± 2.59
15.50	0.73 ± 0.04	11.65 ± 0.69	14.26 ± 0.72^{a}	17.17 ± 1.65
ANOVA *P*-value	0.0014	<.0001	<.0001	<.0001
Regression				
Quadratic *P*-value	0.0001	<.0001	0.0817	<.0001
Broken-line *P*-value	0.0179	0.0571*	0.3417	0.0024

was reported for these species when fish were fed P-deficient diets.

A linear decrease on P utilization was observed in this study. Similar results were reported for gilthead sea bream (Pimentel-Rodrigues & Oliva-Teles, 2001) and rainbow trout (Bureau & Cho, 1999; Coloso *et al*., 2003). The improved P utilization in fish fed low P levels may be a physiological adaptation to the low P content through an increased rate of P absorption as a way to maintain P homeostasis. Increased P absorption has been reported in P-deficient rainbow trout with linear reduction on P digestibility according to the increase of dietary P levels (Rodehutscord *et al*., 2000; Sugiura *et al*., 2000). Although the effect of P levels on

P digestibility has not yet been reported for goldfish, we can assume that the same physiological mechanism may be present in the cyprinids. However, further studies are needed to base our hypothesis.

Whole-body and bone mineral composition is one of the most used parameter to determine bone integrity and P utilization in fish. In this study, no effect of P supplementation on micro mineral composition was observed. However, P supplementation significantly affected whole-body macro mineral composition (Ca, P and Mg) of goldfish. Although a significant effect of P on whole-body micro mineral composition of a cyprinid fish has been reported (Nwanna *et al*., 2010), a highly variable response has been observed among studies.

The dietary requirements for bone or whole-body mineralization in fish is higher than the estimated requirement for growth (Lall, 2002; Yang *et al*., 2006; Zhang *et al*., 2006). However, the requirement for bone mineralization was lower (7.13 g kg^{-1}) than those values estimated for growth (8.2 g kg^{-1}). This low P requirement for goldfish may be related to a low bone density of this ornamental species since several varieties of this fish has thinner bones and increased abdominal mass. However, further studies are needed to determine the bone density mass in goldfish lines and if these factor can influence the mineral requirement for bone or whole-body mineralization.

The results of this study indicate that P is required to maintain normal growth, nutrient utilization, and whole-body mineralization of goldfish fingerlings. Additionally, the use of proper dietary P levels may improve production efficiency, reduce signs of mineral deficiency and reduce P excretion to the water in both commercial production systems and in aquaria used to keep these fish as pets. To the best of our knowledge, this is the first report on mineral requirement for goldfish and further studies are needed to properly comprehend the mineral metabolism in this species.

In conclusion, the use of total P levels between 7.13 and 11.4 g kg^{-1} in goldfish fingerlings diets meets the requirement for maximum growth, feed utilization and proper whole-body mineralization.

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