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Different sources of phosphorus supplementation and its excretion by Nile tilapia juveniles (*Oreochromis niloticus*)

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Abstract. Fish farming causes environmental impacts due to the wastewater produced by fish excreta and unconsumed feed. The differences in phosphorus excretion by Nile tilapia (*Oreochromis niloticus*) juveniles when fed diets composed with different phosphorus sources were evaluated. Six isonitrogenous and isocaloric diets were formulated containing 28% crude protein and 3,000 kcal DE kg^{-1} with levels of 0.8% total phosphorus, and the following ingredients were used as phosphorus source: dicalcium phosphate, meat and bone meal, poultry by-product meal, anchovy meal, tilapia filleting industrial waste, and calcined bone meal . Fish (120 tilapia juveniles) with a mean weight of 94.23 ± 0.28 g and total length of 16.8 ± 0.32 cm were distributed in six 60 L tanks for analysis of the diets digestibility. Diet with dicalcium phosphate presented the lower phosphorus excretion, with retention of 10.05 and excretion of 1.95 kg of phosphorus per ton of tilapia. Diets formulated with lower quality ingredients, such as calcined bone meal and meat and bone meal provided a phosphorus retention of 7.45 and 7.64 and an excretion of 4.55 and 4.36 kg per ton of fish, respectively.

Keywords: aquaculture, carrying capacity, environmental impact

Resumo. Diferentes fontes de suplementação e excreção de fósforo por juvenis de tilápia nilótica (*Oreochromis niloticus*). O cultivo de peixes causa impactos ambientais devido ao efluente produzido que é composto por dejetos e alimento não consumido. As diferenças na excreção de fósforo em juvenis de tilápia nilótica (*Oreochromis niloticus*) alimentados com dietas compostas por diferentes fontes de fósforo foram avaliadas. Seis dietas isocalóricas e isonitrogenadas foram formuladas, contendo 28% de proteína bruta, 3000 kcal de energia digestível, 0,8% de fósforo total, com os seguintes ingredientes utilizados como fonte de fósforo: fosfato bicálcico, farinha de carne e ossos, farinha de vísceras de aves, farinha de anchovas, farinha de resíduos de tilápia e farinha de ossos calcinada. Juvenis de tilápia (120 peixes) com peso médio de 94.23 ± 0.28 g e comprimento total de 16.8 ± 0.32 cm foram distribuídos em seis tanques com volume de 60L para a realização de análises de digestibilidade das dietas. Resultados mostraram que peixes alimentados com a dieta que continha o fosfato bicálcico apresentaram menor excreção de fósforo, com retenção de 10.05 e excreção de 1.95 kg por tonelada de peixe produzido. As dietas formuladas com a farinha de ossos calcinada e a farinha de carne e ossos apresentaram retenção de fósforo de 7.45 e 7.64, e, excreção de 4.55 e 4.36 kg por kg de peixe produzido, respectivamente.

Palavras-chave : aquicultura, capacidade suporte, impacto ambiental

Introduction

Brazil has assumed in its National Policy of Water Resources, that "water is a public good and a limited natural resource with an economic value" which opens a range of social actions. Water is also a natural resource of greatest concern, so the animal production sector has been asked to give its contribution in order to produce with less environmental impact (Spadotto & Ribeiro 2006).

In this context, the sustainable development of agriculture, including fish farming, must preserve the land, water, flora and fauna, to be technically correct, economically viable and socially desirable (Cyrino et al., 2010).

The environmental impact of fish farming mainly results in the enrichment of nutrients such as phosphorus, nitrogen, dissolved solids, organic matter and accumulation of metabolites in fish farming systems that negatively affect growth and survival of the animals, compromising the water quality (Tucker & Hargreaves, 2008).

Thus, the degradation of waste from the fish feed and excreta can contaminate water bodies (Bureau, 2004). Phosphorus (P) is excreted by animals through three pathways: P of the diet that was in inorganic form and was not absorbed; P from endogenous metabolism and cell lyses and also P from phytic acid that was not available in the fish gastrointestinal tract (Witten & Huysseune, 2009).

The increase in the incorporation of P, N (nitrogen) and C (carbon) in the aquatic environment can cause eutrophication (Odum & Barrett, 2007). Therefore, the information about the dietary requirement of P for each fish species and the availability of this nutrient in food is essential for formulating rations to minimize cost and excretion into water (Hakanson, 2005).

The main organic sources of P used in the preparation of feed for fish, which present better availability for animals are the ingredients of animal origin, such as meat and bone meal, poultry by-product meal, fish filleting waste, among others (Gatlin *et al*., 2007; Quintero-Pinto, 2008). Although these meals present a higher amount of available P, its excess as mineral matter present in their composition, may affect its absorption and, resulting in an increased of P excretion in the aquatic environment. This highlights the importance of proper selection and using good quality ingredients.

Among more than 40 potential species for fish farming in Brazil, Nile tilapia (*Oreochromis niloticus* Linnaeus) is the main exotic species current grown in Brazil (Godinho, 2007), its production have increased around 105% in the past seven years, and is currently around 132,000 tons/year, representing 32% of farmed fish produced in Brazil (MPA, 2010). This preference is mainly due to its roughness, great zootechnical performance, handling easiness, appreciable flavor and its meat quality (Faria *et al*., 2002; Boscolo *et al.,* 2005).

Besides that, the Brazilian government is encouraging the practice of intensive aquaculture systems in natural and artificial reservoirs, since there are more than five million hectares suitable for this activity (ANEEL, 2011). Interministerial Normative Instruction No. 07 of 2005 provides that up to 1% of all the federal waters can be used for these purposes, which represents 55,000 hectares for fish production from aquaculture. Considering the management with an average stocking density of 150 kg/m^3/tilapia/year with two harvests per year, it would be possible to achieve a total output of up to 82.5 million tonnes of fish, this value would place Brazil as the second largest aquaculture producer in the world.

However, tilapia cultures use diets with high levels of phosphorus in their formulations, ranging from 1.0 to 3.5%, values that exceed 0.4 to 0.8% of total phosphorus which are recommended as nutritional requirement for this species (Watanabe *et al.,* 2000; Boscolo *et al*. 2005; Quintero-Pinto, 2008; Furuya, 2010; Bueno, 2011). Thus, the fish production increase in Brazilian waters has to be done with proper planning to minimize the phosphorus addition from waste feed and fish waste from these farms.

In this context, fish feeding plays an important role in determining water quality and environmental impact produced by farmed fish waste. The use of least-cost balanced diet and proper food handling are two important requirements for the success in sustainable fish production (Gyllenhammar *et al*., 2011).

Thus, the aim of this study was to evaluate the difference in the total phosphorus (P) excretion by juvenile Nile tilapia (*O. niloticus*) when fed diets containing different sources to P supplementation.

Materials and methods

The experiment was conducted at the Digestibility Laboratory of the Study Group on Aquaculture Management in the West Paraná State

University/Unioeste/ Campus Toledo, under the approval of the Ethics Committee on Animal Experimentation - CEEAAP, Protocol No. 0110.

An homogeneous batch of 480 Nile tilapia (*O. niloticus*) *Chitralada* strain was used with average weight of 94.23 ± 0.28 g and a total length of 16.8 ± 0.32 cm acquired from the same offspring from a juvenile fish production in ponds and raised in the same environmental, nutritional and health conditions. The fish were divided into six cylindrical conical tanks with a capacity of 60 L, fitted with a collection cup at the bottom, where faeces were deposited.

In each collection tank 20 animals were used, and each group was considered an experimental unit. A heater (100 W) was used to maintain the water temperature (25°C) and it was daily measured with a mercury thermometer. Each tank had an aeration system with an air stone attached for air dispersal in order to maintain the dissolved oxygen level at approximately 5.0 mg L^{-1}.

Fish underwent for an adaptation period of fifteen days in the experimental units and a further seven days before going through feeding regimes with each experimental diet.

Experimental design consisted into six treatments and twenty-four replications (diets), conducted in a Latin square scheme. All diets were isonitrogenous and isoenergetic, i.e., 28% crude protein and 3,000 kcal DE kg^{-1} (Table 1). Six diets were formulated with levels of 0.8% of total phosphorus (P) and different ingredients as P source (Table I).

All ingredients were finely grounded and mixed until approximately 0.30 mm. Feeds were extruded obtaining granules with 3 mm in diameter which were dried in a forced air ventilation oven at 55 °C for 24 hours.

Ad libitum feeding was carried out during the adaptation and the experiment period and feces collecting times were: 08:30, 12:00, 13:30, 15:30 and 19:00. Twice a day the tanks were cleaned and 50% of the water volume was exchanged (07:30 and 19:00).

Feces were collected daily from each experimental unit in order to form a composite sample (pool) for each treatment. These were stored in polyethylene containers, identified and frozen at -15 °C for future analysis.

Phosphorus apparent digestibility coefficients (ADC) for tilapia were evaluated by the indirect method, using chromium oxide (Cr_2O_3) as an indicator according to NRC (1993) and incorporated at 0.1% in the feed.

Feed and feces were thawed, dried in forced air circulation at 55 °C for 24 hours, sieved to remove waste and ground for proximate analysis at the Laboratory for Quality Control GEMAq / Unioeste Campus Toledo, Brazil, following the methodology described by AOAC (2000).

The total phosphorus in dry matter and feed mineral matter were determined according to the methodology proposed by Mackreth, Heron, Talling (1978) for total phosphorus, dry matter and mineral followed AOAC (2000).

Chromium oxide (Cr_2O_3) concentration in feed and feces were determined by atomic absorption spectrophotometry according to Kimura and Miller (1957) at the Soil Analysis Laboratory of Maringá State University (UEM), in order to calculate the digestibility.

Phosphorus apparent digestibility coefficients (ADC) in the feed were determined according to the expression proposed by Nose (1960): ADC = 100 - [100 x (%Ir/%If) x (%Nf/ %Nr)], where: ADC= Apparent Digestibility Coefficient (%); %Ir and %If = % feed and feces indicator, respectively; %Nf and %Nr = % of total phosphorus in feed and feces, respectively.

Statistical analysis was performed using Latin square design, with main effects analysis with ANOVA and subsequently Tukey test was applied at 5% significance using the Statistical Analysis System/SAS (1997).

Results

In Table II presents the phosphorus (P) excretion in the aquatic environment per ton of tilapia according to its use in the diet. The dicalcium phosphate showed higher apparent digestibility coefficients (ADC), 83.74% when compared to the other ingredients, showing a better efficiency of this ingredient in organic sources such as meat and bone meal, bone ash and poultry meal. The use of P from this source in the diet resulted in a decrease of 17.32% of P in the aquatic environment when compared to the calcinated bone meal, which has lower availability.

Table II shows that the diets formulated with anchovy meal, tilapia filleting industrial waste and poultry meal obtained values of 77.73, 75.27 and 73.42%, respectively, representing a pollutant potential of 22.27, 26.58 and 24.73%, respectively for P excretion in the water.

Table I. Ingredient inclusion and experimental diet physical-chemical composition with different sources of phosphorus supplementation: T1: dicalcium phosphate (DP) - reference diet; T2: meat and bone meal (MBM), T3: poultry meal (PM); T4: anchovy meal (AM); T5: tilapia filleting industrial meal (TFIM) and T6: calcined bone meal (CBM).

Ingredients	**Treatments**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Soybean meal	51.95	44.98	31.36	26.51	36.02	52.04
Corn grain	39.07	41.25	39.03	40.33	35.10	38.56
Wheat meal	5.00	5.00	12.00	5.00	13.00	5.00
Soybean oil	0.99	1.11	0.00	9.70	0.00	1.14
Vitamin and mineral premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Chromium oxide	0.10	0.10	0.10	0.10	0.10	0.10
DL-Metionine	0.29	0.29	0.24	0.12	0.18	0.29
Dicalcium phosphate (DP)	1.91	0.00	0.00	0.00	0.00	0.00
Meat and bone meal (MBM)	0.00	6.76	0.00	0.00	0.00	0.00
Poultry meal (PM)	0.00	0.00	16.00	0.00	0.00	0.00
Anchovy meal (AM)	0.00	0.00	0.00	17.53	0.00	0.00
Tilapia filleting industrial meal (TFIM)	0.00	0.00	0.00	0.00	15.00	0.00
Calcined bone meal (CBM)	0.00	0.00	0.00	0.00	0.00	2.16
Total	100	100	100	100	100	100
Calculated values[2]						
Starch (%)	25.83	27.19	28.63	26.61	26.20	25.51
Calcium (%)	0.66	0.91	0.80	0.89	1.19	0.89
Digestible energy (kcal/kg)	3000	3000	3000	3000	3000	3000
Crude fiber (%)	4.28	4.00	3.94	2.80	4.00	4.28
Total phosphorus (%)	0.80	0.80	0.80	0.80	0.80	0.80
Lipid (%)	3.22	4.04	4.00	11.53	5.00	3.34
Lysine (%)	1.57	1.53	1.54	1.72	1.70	1.57
Total Met+Cis (%)	1.11	1.11	1.16	0.63	1.16	1.11
Methionine (%)	0.70	0.70	0.71	0.70	0.72	0.70
Crude Protein (%)	28.00	28.00	28.00	28.00	28.40	28.00

[1] Vitamin and mineral premix(Composition /kg of product): Vit. A. - 24.000 UI; Vit. D3 - 6.000 UI; Vit. E - 300 mg; Vit. K3 - 30 mg; Vit. B1 - 40 mg; Vit. B2 - 40 mg; Vit. B6 - 35 mg; Vit. B12 - 80 mg; Folic acid - 12 mg; Calcium Pantothenate - 100 mg; Vit. C - 600 mg; Biotin - 2 mg; Colin - 1.000 mg; Niacin; Fe - 200 mg; Cu - 35 mg; Mn - 100 mg; Zn - 240 mg; I - 1.6 mg; Co - 0.8 mg.
[2]Formulation based on the requirements for species according to Pezzato *et al.* (2002).

Table II. Estimated amount of total phosphorus uptake (retention) and excreted in feces of Nile tilapia (*Oreochromis niloticus*) fed different diets supplemented with phosphorus at 25 °C.

Treatments	**ADC (%)[1]**	**Feed estimated to produce a ton of tilapia[2]**	
		Retention (feed kg P/fish t)[a]	**Effluent (P kg/ fish t)[b]**
T1- Dicalcium phosphate (DP)	83.74%	10.05	1.95
T2 - Meat and bone meal (MBM)	63.64%	7.64	4.36
T3 - Poultry meal (PM)	73.42%	8.81	3.19
T4 - Anchovy meal (AM)	77.73%	9.33	2.67
T5- Tilapia filleting industrial waste (TFIW))	75.27%	9.03	2.97
T6 – Calcined Bone Meal (CBM)	62.09%	7.45	4.55

[1]Apparent digestible coefficient (%) = 100-[100 x (% chromo in feed/chromo in feces) x (% feces total phosphorus /% phosphorus in feed)].
[2]Retention **(a)** Feed estimation to produce one ton of tilapia (feed conversion 1.5:1) x (ingredient percentage in diet) x (digestibility coefficient); Effluent **(b)** Feed estimative to produce one ton of tilapia (feed conversion 1.5:1) x (ingredient percentage in diet) x (100 – apparent digestibility coefficient).

Discussion

According to Ogino *et al.* (1979) P assimilation from mineral sources in the diet is around 90 to 95% for *Cyprinus carpio* (Linnaeus) and *Salmo trout* (Linnaeus). Li and Robinson (1996) in a study for P availability of dicalcium phosphate for channel catfish (*Ictalurus punctatus* Rafinesque) observed a value of 82%. Quintero-Pinto (2008) studying the availability of P during thegrowth, fattening and finishing stages of the *O. niloticus*, obtained a ADC for the dicalcium phosphate of 94.29, 94.95 and 93.84%, respectively and for phosphoric acid of 99.60, 97.31 and 97.16%, respectively.

The lowest value of CDA for the dicalcium phosphate was 74.23% obtained by Miranda *et al.* (2000) for *O. niloticus* with 16 g. Lovell (1978) studying P availability in *I. punctatus* fed diets based on dicalcium phosphate and fishmeal found an ADC for P of 65 and 39%, respectively. Nose and Arai (1978) reported that phosphorus net retention was 61% for tricalcium phosphate, 51% for fishmeal and 19% for rice bran using rainbow trout (*S. trutta trutta* Linnaeus) while for the common carp (*C. carpio carpio* Linnaeus), these figures represented 26.3 and 25% respectively.

However, there is an evidence that the use of better quality ingredients can provide an efficient assimilation of minerals byfish, reducing waste and helping to maintain the carrying capacity of the aquatic environment.

Takamatsu *et al.* (1975) and Shitanda *et al.* (1978) found values of phosphorus availability in fish meal for common carp from 33% and rainbow trout from 60 to 81%, where such differences were correlated to the gastric juices actions.

According to Rosol & Capen (1997), this fact is related to active transport system against using sodium or simply a passive process of diffusion, and absorption of dietary phosphorus may represent 60 to 70% depending on the ingredient availability. Li and Robinson (1996) studying the channel catfish assessed the availability of P in fish meal and found a ADC of 75% for total phosphorus, values similar to our study for tilapia meal (Table 2).

An important factor noted by Watanabe (1988) refers to the difference in processing, quality and bioavailability which exists among ingredients . The author concludes that the rates of absorption of phosphorus in three varieties of salmon fish meal can vary from 30, 65 and 70%.

In this context, the same ingredient (fish meal) had 40% of difference in the pollutant potential to the environment. Cyrino *et al.* (2010) pointed out that the biological value of a particular diet may vary depending on the quality and source of ingredients, use of feed processing techniques, and the interaction between nutrients and dietary supplements. Also, fish feeding habits (carnivore, herbivore, and omnivore) which defines the morphological and physiological species-specific, including stomach pH, intestinal morphology and enzyme activity (Bakke *et al.*, 2010) can be determinant for those values and possible interactions.

Corroborating with this statement, Quintero-Pinto (2008) evaluating phosphorus ADC claims that there are variations among the cultivation phases of *O. niloticus* (25 g initial growth, 250 g grow out and for the 500 g termination) for fishmeal with 52.45, 51.57 and 49.57%, for meat and bone meal with 43.11, 45.48, and 43.77% and for poultry meal with 38.09, 43.39, 44.06%, respectively.

Therefore, when formulating rations, it is important to analyze the ingredients regarding phosphorus sources, because the values obtained from the ADC for the ingredients may vary due to some factors such as ingredient quality, form processing, storage, source, species and growth phase.

The meat and bone meal and calcined bone meal (Table 2) had values above those found by Quintero-Pinto (2008) with an average 44.12% for meat and bones. Guimarães *et al.* (2007) also found values close to 43.66% of ADC obtained in this ingredient. Miranda *et al.* (2000) evaluating *O. niloticus* with average weight of 16 and 86 g had an ADC of 54.59%.

The choice of certain ingredients to be used by the feed industry is directly related to its availability and market price. Table 2 shows that the diets formulated with the highest quality ingredients such as anchovy meal , tilapia filleting industrial waste and dicalcium phosphate showed higher efficiency in the use of phosphorus, providing less excretion in water and hence greater compliance with the requirements for fish.

Unfortunately, the relationship between food cost and pollution potential are currently not considered. In this context, most companies in fish nutrition business have their focus at the production cost, and thus choose alternative sub-products, such as animal meal with poor quality or substitute with alternative sources, such as vegetable flours with low phosphorus availability.

Cao *et al.* (2008) pointed out that the P ingested from vegetable meals in the form of phytate by monogastric animals is rarely available due to the lack of phytases, therefore, adding these enzymes in the diets can increase P availability. Thus, when using vegetable sources, the use of phytase may be a strategy to increase P absorption efficiency and decrease its excretion, helping to reduce P waste (Kumar *et al.,* 2011). As shown in a study by Furuya (2001), which evaluated the ADC for P in fish meal, corn, wheat bran and soybean meal for Nile tilapia, distinct values were obtained, being 49.6, 50.0, 29.4, and 47.7%, respectively.

Steffens (1987) mentions that vegetable-based diets for common carp have assimilation around 40%. Gonçalves (2007) evaluating the ADC of P in diets for 100 g tilapia, found assimilation in corn gluten meal of 22.20%, 22.30% in soybean meal and 52.90% in cotton seed meal. Guimarães *et al. (*2007) in a study with 86 g *O. niloticus* verified that the ADC of P was 26.96% for soybean meal and 3.51% for cottonseed meal.

The difference in Passimilation between plant and animal sourcesbecomes evident, considering that to produce one ton of tilapia with an average conversion of 1.5, it needs to besupplied 1,500 kg of feed, considering the phosphorus requirement of 0.8% for this species (Furuya, 2010). When using the soybean meal that has an apparent availability of 35.13% (Miranda *et al*., 2000), it was registered a P excretion ofapproximately 7.78 kg per ton of tilapia. To produce the same amount of fish, using anchovy meal, Pexcretion observed was 2.67 kg per ton of fish (Table 2)..

However, good manufacturing practices and diets management are essential in aquaculture in order to promote the activity with environmental sustainability, since an erroneous practice can cause environmental impact in the aquatic environment (Pillay, 2007). Thus, the use of diets with the highest quality ingredients with the greatest availability of minerals is essential to minimize the environmental impact of phosphorus coming from the feed for fish culture, since in the medium and long term the economic sustainability will have to incorporate the maintaining costs to keep the environmental quality at the farming systems.

Conclusions

Feed ingredients quality and digestibility assessment are an effective technique to determine phosphorus concentration and waste released into the aquatic environment.

The diet with lowest excretion rate in the aquatic environment was dicalcium phosphate treatment, which showed a 10.05 retention and excretion of 1.95 kg of phosphorus per ton of tilapia. Diets formulated with lower quality ingredients such as calcinated bone meal and meat and bone meal provide a total phosphorus retention of 4.55 and excretion of 4.36 kg per ton of fish, respectively.

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