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ANIMAL PRODUCTION

Stability and phosphorus leaching of tilapia feed in water

Guilherme Wolff Bueno[1,2*] Bruno Olivetti de Mattos[3] Dacley Hertes Neu[4] Fernanda Seles David[2] Aldi Feiden[5] Wilson Rogério Boscolo[5]

[1]Curso de Engenharia de Pesca, Universidade Estadual Paulista (UNESP), Campus de Registro, 11900-000, Registro, SP, Brasil. E-mail: gwolff@reitoria.unesp.br. *Corresponding author.
[2]Centro de Aquicultura da Unesp (CAUNESP), Universidade Estadual Paulista (Unesp), Jaboticabal, São Paulo, Brasil.
[3]Programa de Pós-graduação em Aquicultura, Universidade Nilton Lins, Manaus, AM, Brasil.
[4]Faculdade de Ciências Agrarias (FCA), Universidade Federal da Grande Dourados (UFGD), Dourados, MS, Brasil.
[5]Grupo de Estudos de Manejo na Aquicultura (GEMAQ), Universidade Estadual do Oeste do Paraná (UNIOESTE), Toledo, PR, Brasil.

ABSTRACT: *The present research aimed to investigate the stabil¬¬ity of pellets and phosphorus leaching of diets formulated for juveniles of Nile tilapia (Oreochromis niloticus), with different sources of phosphorus and different exposure times in water. Six diets were elaborated by varying the source of phosphorus (1 – dicalcium phosphate (DP); 2 – meat and bone meal (MBM); 3 – poultry meal (PM); 4 – anchovy meal (AM); 5 – tilapia filleting industrial meal (TM); 6 – calcined bone meal (CBM)) and, then, were submitted to four exposure times in water (5, 10, 15 and 20 minutes), with three replicates. Thus, 72 aquariums of 30–liters were used, each being an experimental unit. All diets were evaluated for electrical conductivity of water, turgidity of pellets, mineral matter leaching, flotation of pellets, and total phosphorus leaching. Only turgidity and flotation of pellets varied with the different sources of phosphorus in the diets. The MBM diet had the highest turgidity of pellets. The PM, AM, and CBM diets had the highest flotation of pellets. The total phosphorus leaching had a linear effect with the increase of the exposure time, showing a greater release of phosphorus in the water with increase of exposure time. Data showed that PM, AM, and CBM diets had less potential impact on the aquatic environment. Conversely, the TM diet has greater polluting potential. These results showed that diets formulated with different sources of phosphorus exhibit distinct actions in the water, providing different effects on the fish culture environment.*
Key words: *aquaculture, aquafeed, environmental impact, phosphorus leaching, water quality.*

Estabilidade e lixiviação do fósforo da ração de tilápia na água

RESUMO: *O presente trabalho tem como objetivo investigar a estabilidade de pellets e a lixiviação do fósforo na água proveniente de diferentes dietas formuladas para juvenis de tilápia do Nilo (Oreochromis niloticus), considerando distintas fontes de fósforo e diferentes tempos de exposição na água. Para tanto, foram elaboradas seis dietas com variação da fonte de fósforo (1: fosfato dicálcico (DP); 2: farelo de carne e ossos (MBM); 3: farelo de aves (PM); 4: farelo de anchova (AM); 5: farelo industrial de filetagem de tilápia (TM); 6: farelo de osso calcinado (CBM)), as quais foram submetidas a quatro tempos de exposição em água (5, 10, 15 e 20 minutos), com três repetições. Utilizaram-se 72 aquários de 30 litros, sendo cada um deles uma unidade experimental. A água dentro dos aquários foi mantida sob constante aeração e temperatura ao redor de 25 °C. Todas as dietas foram avaliadas quanto à condutividade elétrica da água, turgidez, lixiviação de matéria mineral, flotação de pellets e lixiviação total do fósforo. Apenas a turgidez e a flutuação dos pellets variaram com as diferentes fontes de fósforo nas dietas. A dieta MBM apresentou a maior turgidez de pellets. As dietas PM, AM e CBM apresentaram a maior flutuação de pellets. A lixiviação do fósforo total teve um efeito linear com o aumento do tempo de exposição, resultando em maior liberação de fósforo na água. A lixiviação de matéria mineral apresentou interação entre fontes de fósforo e tempos de exposição das dietas, com efeito linear para a dieta TM. As dietas PM, AM e CBM apresentam as menores concentrações de efluentes em relação a dieta TM. Esses resultados revelaram que dietas formuladas com diferentes fontes de fósforo apresentam ações distintas na água em relação ao potencial poluidor.*
Palavras-chave: *aquicultura, aquafeed, impacto ambiental, lixiviação do fósforo, qualidade da água.*

INTRODUCTION

Aquaculture is expanding worldwide, especially in the last 20 years, and this trend is likely to continue (FAO, 2017). In 2016, the global production of fisheries and aquaculture reached approximately 200 million tonnes of fish, of which 47% came from aquaculture (FAO, 2018). This activity is an important source of high quality protein, mainly in developing countries that need to increase food production for local consumption (EL-GAYAR & LEUNG, 2000). In addition, it generates benefits for the regional economies in the form of employment and income throughout the production chain (ROSS et al., 2011; MACFADYEN et al., 2012; RORIZ et al., 2017), constituting an important alternative for the populations (ABERY et al., 2005).

Increasing production of fish in feed lot systems has raised concerns about the environmental impact that diets can cause in the aquatic environment

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(BUENO et al., 2016; HARDY, 2010). These diets, when in contact with the water, lose nutrients and, when not consumed, raise nutrient concentrations in the environment (SOARES-JÚNIOR et al., 2007; OLIVEIRA-SEGUNDO et al., 2013). Among the nutrients lost from diets, phosphorus is the most critical, since it influences directly the eutrophication process (Martin et al., 2010; HAN et al., 2016; Wang et al., 2016).

Phosphorus is a key element because it is essential for animal nutrition (STEFFENS, 1989). This nutrient is responsible for mineralization of the bone matrix (Kay et al., 1964; MCDOWELL, 1992; FURUYA et al., 2007) and it is involved with metabolic functions (BAEVERFJORD et al., 1998; CHAVEZ-SANCHEZ et al., 2000; LALL, 2002), cellular differentiation (LOVELL, 1998) formation of phospholipids (SARGENT et al., 2002) and osmotic balance (BARZEL, 1971). Nevertheless, in excess, it is not assimilated by the raised organisms, becoming available in the environment (LUPATSCH & KISSIL, 1998; BUENO et al., 2008; YUAN et al., 2011). Phosphorus concentrations in the water and in the sediment increases according to the amount of feed supplied (DAVID et al., 2017). As there is little phosphorus naturally in the water, such element in excess can cause eutrophication, which increases the biochemical demand of oxygen and, thus, reduces the availability of oxygen in the environment, causing the death of aquatic organisms by hypoxia (TUNDISI & TUNDISI, 2008).

The degree of eutrophication caused by lost nutrients is related to nutritional management. Alternatives to mitigate this problem are being investigated, such as the use of binders to reduce leaching and increase the stability of diets in the water (CANTELMO et al., 2008; PEZZATO et al., 1995a, 1995b) and the search for adequate concentrations of nutrients for better fish nutrition (Furuya et al., 2001; Gonçalves et al., 2007). Likewise, the search for sources with better indices of nutritional bioavailability for diets formulation (BUREAU & HUA et al., 2010) and the understanding of their pollutant potential based on a coefficient of digestibility of ingredients (BUENO et al., 2012) can provide better productive performance and reduce the excretion of nutrients in the aquatic environment. Nonetheless, no studies have addressed information on the amount of phosphorus lost to the environment when considering different sources of this element in the diets. In this context, the aim of this research is to investigate the stability of pellets and phosphorus leaching of diets formulated for juveniles of Nile tilapia, considering different sources of phosphorus and different exposure times in water.

MATERIALS AND METHODS

Experimental diets

Six diets were elaborated with the following sources of phosphorus: 1 – dicalcium phosphate (DP); reference diet, 2 – meat and bone meal (MBM), 3 – poultry meal (PM), 4 – anchovy meal (AM), 5 – tilapia filleting industrial meal (TM), and 6 – calcined bone meal (CBM). Diets were formulated according to the nutritional recommendations for Nile tilapia juveniles (Furuya, 2010; NRC, 2011) and are given in table 1. All diets were prepared as isonitrogenous and isoenergetic. In addition, all diets contained 0.8% of phosphorus, being differentiated by the ingredients as source of this mineral. Each diet was supplemented with an equal quantity of vitamins and minerals.

The ingredients were ground in hammer mills with 0.33mm diameter sieve, mixed, moistened with 22% water, homogenized and extruded in a professional extruder (Ex-Micro® extruder, ExTeec Company, Ribeirão Preto, Brazil) at approximately 90 ºC to obtain 3-mm-diameter pellets. After this process, the pellets were dried for approximately 12 hours in a forced circulation oven at 55 °C. Then, pellets were stored in a freezer (5 °C) until use.

Experimental design

All of six diets were submitted to four exposure times in the water (5, 10, 15 and 20 minutes), with three replicates. Thus, the experiment was performed in 72 aquariums of 30-liters, each being an experimental unit. The water inside aquariums was kept under constant aeration and temperature ~25 °C.

In order to characterize the potential of environmental pollution in the different exposure times, the diets were evaluated in relation to the electrical conductivity of water (C), turgidity of pellets (T), mineral matter leaching (MML), flotation of pellets (F), and total phosphorus leaching (PL). Electrical conductivity of the water was measured in situ using a multisensor probe (Hanna Instruments® HI 991301, São Paulo, Brazil). Flotation and stability of pellets were measured according to Pezzato et al. (1995a), mineral matter and total phosphorus leaching were measured according to Pezzato et al. (1995b), and total phosphorus and mineral matter were determined, respectively, according to the methodology proposed by Mackereth et al. (1978) and AOAC (2012).

Data analysis

Data were analyzed using PROC MIXED procedure of SAS (version 9.2) with fixed effects

Table 1 - Ingredients inclusion and physical-chemical composition of the experimental diets with different sources of phosphorus: (1) dicalcium phosphate (DP) - reference diet, (2) meat and bone meal (MBM), (3) poultry meal (PM), (4) anchovy meal (AM), (5) tilapia filleting industrial meal (TM), and (6) calcined bone meal (CBM).

Ingredients	Treatments					
	DP	MBM	PM	AM	TM	CBM
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	1.91	-	-	-	-	-
Meat and bone meal	-	6.76	-	-	-	-
Poultry meal	-	-	16.00	-	-	-
Anchovy meal	-	-	-	17.53	-	-
Tilapia filleting meal	-	-	-	-	15.00	-
Calcined bone meal	-	-	-	-	-	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 of Nile tilapia juveniles (*Oreochromis niloticus*) (FURUYA, 2010; NRC, 2011).

represented by phosphorus sources in the diets, exposure times of diets in the water and the interaction between them. In the case of interaction effects, data were subjected to the SLICE command in order to compare the mean of phosphorus sources using Tukey's test. Regarding the exposure times of diets in the water, the mean was compared using unfolding orthogonal contrasting in effects linear and quadratic. The statistical procedure was conducted with 0.01 as the critical probability level for Type I error.

RESULTS

Regarding the sources of phosphorus in the diets, no significant differences ($P>0.01$) were reported for mineral matter leaching and total phosphorus leaching. The electrical conductivity of water presented statistical differences among treatments, but it is not possible to detect relevant information. The turgidity and flotation of pellets; however, varied with the different sources of phosphorus in the diets. The diet formulated with meat and bone meal (MBM) had the highest turgidity of pellets. Diets formulated with poultry meal (PM), anchovy meal (AM), and calcined bone meal (CBM) had the highest flotation of pellets.

Regarding the time of exposure of the diets in the water, only electrical conductivity of the water was not affected by the evaluated treatments. The total phosphorus leaching had a linear effect ($P<0.01$), thus, there was greater release of phosphorus in the water with the increase of the exposure time. Mineral matter leaching had an interaction between phosphorus sources and exposure times of the diets (Tables 2 and 3), with a linear effect (P<0.01) for the diet formulated with tilapia filleting industrial meal (TM). Thus, with the increase of the times of exposure, there was more leaching of the mineral matter in the water with the diet formulated with tilapia flour.

DISCUSSION

Diets formulated according to nutritional recommendations for juveniles of Nile tilapia, but with different sources of phosphorus in their formulation, affect the aquatic environment in different ways. Among the evaluated diets, those formulated with poultry meal (PM), anchovy meal (AM), and calcined bone meal (CBM) had less potential impact on environment, as they presented lower values of turgidity and higher values of flotation of the pellets. Conversely, the diet formulated with tilapia filleting industrial meal (TM) has a greater polluting potential because of the interaction between the source of phosphorus and the times of exposure in the water, showing the most impacting results of mineral material leaching.

When the interaction with the exposure time was not considered, the phosphorus sources of diets did not present significant differences (P>0.01) regarding mineral matter leaching and total phosphorus leaching (Table 2). Nevertheless, phosphorus values ranged from 0.17 to 0.21 mg.L-[1], which is higher than recommended by CONAMA Resolution nº 357/2005. For aquaculture practice, this Resolution establishes phosphorus limits at 0.030 mg.L-[1] for lentic environments and 0.050 mg L-[1] for intermediate environments. In the present research, due to the experimental conditions, we did not consider all the variables that can interfere in the production cycle. Considering factors such as stocking density, feed frequency, water temperature, media interactions, tributary inflow and effluent output, this condition can be aggravated, depending on the intensity of the system (MONTE-LUNA et al., 2004; KARAKASSIS et al., 2013; KLUGER et al., 2016). Moreover, in this research, the diets were processed following recommendations for the structure and composition of diets, as well as for pellets processing. It is important to emphasize that when these quality standards are disregarded, the mineral matter and total phosphorus leaching may be even more representative (NRC, 2011). This shows the importance, and necessity, of formulating and providing nutritional and environmental quality diets.

Water quality is affected by the exposure time of all diets in the water, but to different degrees. In general, increasing exposure time leads to a change in water quality. The linear effect of total phosphorus leaching with the increase of the exposure time showed a greater release of phosphorus in the water over time. The interaction between mineral matter leaching, phosphorus sources and exposure times of diets, with a linear effect (P<0.01) for the diet formulated with tilapia flour (TM), showed that, with the increase of the exposure time, the diet based on tilapia by-products releases more mineral matter into

Table 2 - Environmental pollution parameters regarding pellets stability and phosphorus leaching obtained during the different exposure times of the experimental diets*: Electrical conductivity of water (C), turgidity of pellets (T), mineral matter leaching (MML), flotation of pellets (F), and total phosphorus leaching (PL).

Parameters	----------------------Treatments----------------------						------------Time (min)----------					----------------*P* value--------------		
	DP	MBM	PM	AM	TM	CBM	5	10	15	20	SEM	Phosphorus (P)	Time (T)	P*T
C ($\mu.cm^{-1}$)	66.3^{a}	69.5^{b}	67.6^{ab}	68.7^{ab}	69.7^{b}	68.7^{ab}	67.5	68.0	68.5	69.6	0.63	0.01	0.07	0.09
T (%)	8.41^{b}	39.8^{d}	3.08^{a}	0.83^{a}	22.2^{c}	3.66^{a}	12.7	12.1	14.1	13.1	1.00	<0.01	0.47	0.66
MML (%)	1.40	1.29	1.00	0.96	1.87	1.74	1.19	1.38	1.41	1.52	0.005	0.31	0.01	0.01
F (%)	91.5^{c}	60.1^{b}	96.9^{d}	99.1^{d}	77.7^{a}	96.3^{d}	87.2	87.8	85.8	86.8	1.01	<0.01	0.47	0.66
PL ($mg.L^{1}$)	0.21	0.18	0.17	0.19	0.19	0.20	0.16	0.17	0.20	0.22	0.13	0.11	<0.01[1]	0.06

Different letters in the same line indicate significant differences among treatments (*P*<0.01).

*Experimental diets: (1) dicalcium phosphate (DP) - reference diet, (2) meat and bone meal (MBM), (3) poultry meal (PM), (4) anchovy meal (AM), (5) tilapia filleting industrial meal (TM), and (6) calcined bone meal (CBM).

1 Contrast: linear P<0.01 and quadratic p=0.43.

Table 3 - Environmental pollution analysis - mineral matter leaching (%) - obtained during the different exposure times of the experimental diets*.

Time (min)	Treatments						P value
	DP	MBM	PM	AM	TM	CBM	
5	1.31	1.17	0.81	0.80	1.45	1.62	0.31
10	1.35	1.31	1.00	1.05	1.85	1.72	0.29
15	1.46	1.30	0.92	0.98	2.19	1.64	0.20
20	1.46	1.39	1.30	1.01	2.00	1.98	0.42
P value	0.79	0.84	0.59	0.57	< 0.01[1]	0.83	

*Experimental diets: (1) dicalcium phosphate (DP) - reference diet, (2) meat and bone meal (MBM), (3) poultry meal (PM), (4) anchovy meal (AM), (5) tilapia filleting industrial meal (TM), and (6) calcined bone meal (CBM).

the water. Overall, these data showed that nutrient loss occurs according to the increased exposure time of the diets in the water. Thus, the performance of reared animals may be affected by the loss of key nutrients, such as phosphorus, compromising metabolic and physiological functions (ROY & LALL, 2003; MAI et al., 2006; YE et al., 2006; FURUYA et al., 2007). In addition to the effects linked to animal nutrition, the loss of dietary phosphorus to water can cause environmental impacts. Phosphorus in water becomes an important source for the growth of microalgae and plants (LAMBERS et al., 2008; MCDOWELL et al., 2015; Ni & Wang, 2015) and, in excess promotes eutrophication (AHLGREN et al., 2006; CONLEY et al., 2009; MEINIKMANN et al., 2015).

The electrical conductivity of the water does not seem to be affected differently by the evaluated diets. Considering values obtained for each diet along with the exposure times in the water, no significant differences were reported and all treatments maintained the electrical conductivity within the established standard for aquaculture (BOYD, 1980). This parameter is environmentally important because it represents a way to assess the availability of nutrients in aquatic ecosystems and environmental pollution. In the case of high values, the electrical conductivity of the water is related to the degree of decomposition or dissolved solids and, in the case of reduced values, it indicates a marked primary production (BOYD, 1980; TUNDISI & TUNDISI, 2008).

The turgidity and floating of pellets are also important parameters to be evaluated, since they may reflect on water quality. In the present work, diets formulated with poultry meal (PM), anchovy meal (AM), and calcined bone meal (CBM) represent lower environmental risk, as they presented lower turgidity and higher flotation of the pellets. Thus, it is possible to infer that diets present different responses when formulated with different sources of phosphorus. Furthermore; although, the diets are isonitrogenous and isoenergetic, the stability and floating of pellets vary with the lipid content (NRC, 2011).

In aquaculture, feed supply is the major driver that affects the phosphorus budget in production systems (DAVID et al., 2017). Thus, manufacturing practices and productive management are fundamental to promote environmental sustainability (Patel and Yakupitiyage, 2003; EL-SAYED, 2013). Erroneous practice can compromise and cause negative impact on the aquatic environment (PILLAY, 2007; BHUJEL, 2013). Thus, the use of diets formulated with ingredients that present greater availability of phosphorus is fundamental to minimize such impact (LIEBERT & PORTZ, 2005; ROY & LALL, 2003), since it improves the digestibility satisfactory (Miranda et al., 2000; FURUYA et al., 2001) and avoids high rates of excretion or leaching of this nutrient environment (BUENO et al., 2016; LUPATSCH & KISSIL, 1998).

CONCLUSION

Diets formulated with different sources of phosphorus exhibited distinct actions in the water, providing different effects on the fish culture environment. Data showed that diets formulated with poultry meal (PM), anchovy meal (AM), and calcined bone meal (CBM) had less potential impact on the aquatic environment. Conversely, the diet formulated with tilapia filleting industrial meal (TM), which is used by the industry due to the by-products of filleting, indicated a greater polluting potential. These results; however, should be analyzed together with the factors that interfere with the production cycle. Future research should focus on the commercial application of the exploration of sources used in the formulation of aquatic feeds.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Protocol nº. 0110

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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