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ANIMAL PRODUCTION

Available phosphorus from phosphate extracted from swine wastewater for poultry

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ABSTRACT: This research evaluated phosphorus availability, from phosphate extracted from swine wastewater, in broilers. Phosphorus inorganic contaminants were determined. Afterward, an experiment was carried out with 2,520 broilers, divided in randomized blocks, 9 treatments (0, 0.5, 1.0, 1.5, and 2.0g/kg P from phosphate extracted from swine wastewater and 0.5, 1.0, 1.5, and 2.0g/kg P from dicalcium phosphate), 10 repetitions and 28 birds per experimental unit. The animals' age was from 1 to 14 days old, which were housed in boxes and fed *ad libitum* (water and feed). At 14 days, 3 birds per experimental unit were slaughtered to evaluate tibia-breaking strength. Data were submitted to analysis of variance and regression. Phosphorus biological availability from the commercial source was calculated by the ratio of the regression coefficients, considering the phosphorus from dicalcium phosphate as 100% available. The inorganic contaminants showed that concentrations in phosphate extracted from swine wastewater in relation to dicalcium phosphate are similar or at lower concentration levels. The phosphorus availability of 32.53% was observed for simple linear equation and 31.38% for multiple linear equation. Phosphate advantages from swine wastewater are reflected in the environmental issue, that is, free of contamination (inorganic metals) and less amount disposed in the environment. The outcomes of this research indicated no pathogens (*Salmonella* sp. and *E. coli*) in the phosphate extracted from swine wastewater applied in broilers, and phosphorus average availability of 31%.
Key words: contaminants, swine wastewater, phosphorus availability, phosphate, environment.

Fósforo disponível de fosfato extraído de efluentes de suinocultura para frangos de corte

RESUMO: Esta pesquisa objetivou avaliar a disponibilidade do fósforo, de fosfato extraído de efluentes da suinocultura, em frangos de corte. Foram determinados os contaminantes inorgânicos do fosfato e posteriormente, foi realizado experimento com 2520 frangos de corte divididos aleatoriamente em blocos casualizados, com nove tratamentos (0; 0,5; 1,0; 1,5 e 2,0g/kg P de fosfato extraído de efluentes da suinocultura e 0,5; 1,0; 1,5 e 2,0g/kg P de fosfato bicálcico), 10 repetições e 28 aves por unidade experimental. A idade das aves foi de 1 a 14 dias, as quais foram alojadas em boxes, com água e ração à vontade. Aos 14 dias, três aves por unidade experimental foram abatidas para avaliação da força de quebra da tíbia. Os dados foram submetidos à análise de variância e regressão, calculando-se a disponibilidade biológica do fósforo do fosfato bicálcico pela relação dos coeficientes de regressão, considerando o fósforo do fosfato bicálcico como 100% disponível. Os resultados para determinação de contaminantes demonstraram que concentrações do fosfato extraído de efluente da suinocultura em relação ao fosfato bicálcico são similares ou em níveis inferiores. Foi observada disponibilidade de 32,53% do fósforo de efluente da suinocultura para equação linear simples e 31,38% para equação linear múltipla. Os ganhos com fosfato oriundo de efluentes da suinocultura se refletem na questão ambiental, ou seja, sem contaminação por metais inorgânicos e menores quantidades eliminadas para o meio-ambiente. Portanto, o fosfato extraído de efluentes da suinocultura aplicado em frangos possui baixo risco de patógenos (*E. coli* e *Salmonella* sp.) e disponibilidade média de 31%.
Palavras-chave: contaminantes, efluentes da suincultura, disponibilidade de fósforo, fosfato, meio ambiente.

INTRODUCTION

Brazil has prominence in agribusiness due to its economic and social importance; however, pig farming is one of the greatest polluting activities in livestock, because it generates high quantity of effluents. Indeed, these effluents are rich in nutrients, especially nitrogen and phosphorus, which can cause environmental imbalance when disposed in high amount in the environment.

The process to remove phosphorus is an important alternative to minimize the impacts caused by the lack of management and control of effluents and, to the extent possible, add value to these residues. To perform phosphorus removal, many processes are proposed, such as chemical, physical, and biological ones. In this context, the chemical removal process is considered viable and of low cost and employs calcium hydroxide (hydrated lime) as a biological post treatment, generating calcium phosphate, which

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can later be used as a fertilizer or as an animal feed ingredient (FERNANDES et al., 2012).

To formulate animal diets, phosphorus may be withdrawn from animal, plant or inorganic sources (MIRABILE et al., 2020; TRAIRATAPIWAN et al., 2018). For swine and poultry, phosphorus is an essential nutrient and one of the most expensive feed components, considering that it is directly associated with bone formation (80% of phosphorus is found in skeletal composition) and in the use and transport of energy. It must be highlighted that the current sources of phosphorus are finite, with the main supplementary source being dicalcium phosphate, which is an inorganic mineral source (PINHEIRO et al., 2011).

The phosphorus requirement to increase animal performance is below the maximum level for bone development. Thus, in situations where the diet is low in phosphorus, animal physiological needs are met by the phosphorus mobilization from the bones, which can lead to growth with low bone resistance. According to BÜNZEN et al. (2012), phosphorus from dicalcium phosphate is considered 100% available, which is why it is being widely used in animal feed formulation worldwide. Thus, the objective of the present research was to evaluate phosphorus availability, from phosphate extracted from swine wastewater, in broilers.

MATERIALS AND METHODS

Sample collection was carried out at the Embrapa Swine and Poultry Treatment Station in Concórdia, Santa Catarina, Brazil. The samples consisted of: (i) phosphate extracted from swine wastewater after precipitation with hydrated lime and open-air drying, (ii) hydrated lime, (iii) dicalcium phosphate. The samples were taken to the Laboratory of Industrial and Environmental Chemical Analysis (LAQIA) at the Federal University of Santa Maria, in closed plastic packages and stored in a dry place, at room temperature. Approximately 300 mg of each sample (n = 6) were dissolved in 3 mL HNO_3 at 14 mol/L in polypropylene vials (50 mL). After weighing, the acid was added, and the flask stirred (for about 30 s) manually. After complete solubilization, the volume was adjusted with ultra-pure water (up to 30 mL) to later determine the inorganic constituents.

The determinations of Al (aluminum), Ca (calcium), Cu (copper), Fe (iron), Mg (magnesium), Mn (manganese), P (phosphorus) and Zn (zinc) were carried out by optical emission spectrometry with inductively coupled plasma. An Optima 4300 DV simultaneous spectrometer, equipped with a cyclonic nebulizer chamber and a concentric nebulizer was used. The plasma power was 1400 W, and the argon flow rates were 15 L/min (plasma), 0.7 L/min (nebulizer) and 0.2 L/min (auxiliary). The wavelengths were 396.15 nm (Al), 396.85 nm (Ca), 324.75 nm (Cu), 238.20 nm (Fe), 285.21 nm (Mg), 257.61 nm (Mn), 177.43 nm (P), 290.88 nm (V) and 213.86 nm (Zn).

The determinations of As (arsenic); Cd (cadmium); Co (cobalt); Cr (chromium) Mo (molybdenum), Ni (nickel); Pb (lead) and V (vanadium) were accomplished by mass spectrometry with inductively coupled plasma, using a spectrometer model Elan DRC II, equipped with concentric nebulizer, cyclonic nebulization chamber and torch with a 2 mm internal quartz injector. The power was 1400 W, and the argon flow rate was 15.0, 1.2 and 1.15 L/min, for plasma, auxiliary and nebulization gas, respectively. Measurements were made using the 75 As, 114 Cd, 59 Co, 53 Cr, 98 Mo, 58 Ni and 207 Pb isotopes.

The determination of Hg (mercury) was made by mass spectrometry with plasma inductively coupled by flow injection under cold steam, using the same equipment, with the coupling of a flow injection system and cold steam generation.

For the field experiment, 2,520-day-old male broilers of the Cobb strain were used. The experimental design was in randomized blocks, with nine treatments: 0, 0.5, 1.0, 1.5, and 2.0g/kg P from phosphate extracted from swine wastewater and 0.5, 1.0, 1.5, and 2.0g/kg P from dicalcium phosphate and ten replicates, with 28 birds per experimental unit.

The 1-14 days old birds were housed in boxes with wood shavings, and water and food were provided *ad libitum*. During the experimental period, the temperature and relative humidity were measured inside the shed by using six data loggers, distributed through the boxes the values were on average 22.7 °C and 56.7% respectively.

The basal diet was based on corn and soybean meal, according to recommendations by ROSTAGNO et al. (2011), except for P. The experimental diets were made by mixing the basal diet with tested phosphates or kaolin (used as inert) to make up 100% of the diet, and they are shown in table 1. The phosphate extracted from swine wastewater contained 2.89g/kg Ca, 30g/kg P, and 18.2g/kg Mg.

At 14 days of age, three birds were removed per experimental unit. The birds were slaughtered, then the tibia (n = 270) was removed. Afterward, the dry tibia-breaking strength was evaluated to determine the available phosphorus.

Microbiological assays were performed according to the Bacteriological Analytical Manual of the Food and Drug Administration. *E. coli*

Table 1 - Composition of the experimental diets.

Ingredients (g/kg)	Control	-------------------------PESW------------------------				-----------------Dicalcium phosphate---------------			
	0.00	0.5	1.0	1.5	2.0	0.5	1.0	1.5	2.0
Corn	419.39	419.39	419.39	419.39	419.39	419.39	419.39	419.39	419.39
Soybean meal	404.81	404.81	404.81	404.81	404.81	404.81	404.81	404.81	404.81
Soybean oil	70.87	70.87	70.87	70.87	70.87	70.87	70.87	70.87	70.87
Kaolin	70.42	52.82	35.21	17.61	0.00	67.63	64.84	62.05	59.25
Dicalcium phosphate	0.00	0.00	0.00	0.00	0.00	2.79	5.58	8.38	11.17
PESW	0.00	17.61	35.21	52.82	70.42	0.00	0.00	0.00	0.00
Limestone	20.11	20.11	20.11	20.11	20.11	20.11	20.11	20.11	20.11
Salt	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95	4.95
DL-Methionine	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02	3.02
Adsorbent	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Premix[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
L-Lysine	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Choline chloride, 70%	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Coban 200	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Mineral premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Threonine	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Tylan 40	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
BHT (antioxidant)[3]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
AME, kcal/kg	2985	2985	2985	2985	2985	2985	2985	2985	2985
Lys dig, g/kg	12.19	12.19	12.19	12.19	12.19	12.19	12.19	12.19	12.19
P, g/kg	3.69	4.19	4.69	5.19	5.69	4.19	4.69	5.19	5.69

1 Provided per kg of product - Retinol: 9 000 000 UI; Cholecalciferol: 2 500 000 UI; α-ocopherol: 20 000 mg; Menadione: 2 500 mg; Thiamin: 1 500 mg; Riboflavin: 6 000 mg; Pyridoxine: 3 000mg; Cyanocobalamin: 12 000 mg; Pantothenic acid: 12 g; Niacin: 25 g; Folic acid: 800 mg; Biotin: 60 mg; Se (as sodium selenite): 250 mg.

2 Provided per kg of product - Cu (as copper sulfate): 20 g; Fe (as ferrous sulfate): 100 g; Mn (as manganese oxide): 160 g; Co (as cobalt sulfate): 2 000 mg; I (as calcium iodate): 2 000 mg; Zn (as zinc oxide): 100 g.

3 Butylated Hydroxytoluene.

assay followed one protocol (FOOD AND DRUG ADMINISTRATION, 2020) and *Salmonella* sp. assessment followed another (FOOD AND DRUG ADMINISTRATION, 1992).

The data were submitted to analysis of variance (ANOVA) and simple and multiple linear regression using SAS software (SAS INSTITUTE INC, 2012). Then, phosphorus biological availability was calculated by the ratio of the regression coefficients, considering the phosphorus from dicalcium phosphate as 100% available.

RESULTS AND DISCUSSION

The results (Table 2) of inorganic contaminants in phosphate extracted from swine wastewater had lower or similar concentration levels when compared to dicalcium phosphate.

In comparison with hydrated lime, it is possible to verify that there is no contamination when using this raw material for the phosphate precipitation process. Therefore, when it comes to phosphorus removal processes, it appears that the use of hydrated lime (for chemical processing) becomes the best alternative to be used (FERNANDES et al., 2012).

Therefore, it is important to be aware of the number of inorganic contaminants in wastewater by studying phosphorus availability. First, because a large amount of phosphorus is disposed into the environment through wastewater and second because calcium phosphate is obtained as a final product, which can be reused as a fertilizer or, after processing, to produce phosphate concentrates, used as ingredients in animal feed (VANOTTI et al., 2003).

The results for tibia-breaking strength are shown in table 3. Phosphorus availability was

Table 2 - Determination of minerals (µg/g) in phosphate extracted from swine wastewater (PESW), dicalcium phosphate and hydrated lime.

Element	----------------PESW----------------		-------Dicalcium phosphate-------		-----------Hydrated lime-----------	
	Average	CV (%)	Average	CV (%)	Average	CV (%)
Al	359	3.0	905	1.6	1409	5.5
At	0.4	16.9	4.1	3.3	1.4	8.2
Cd	< 0.25		5.92	3.5	< 0.25	
Co	1.6	4	3	3.7	1.7	2.6
Cr	< 0.5		110	3.6	< 0.5	
Cu	40.7	1.9	18.7	1.8	< 0.5	
F	103	2.0	1019	7.0	496	4.0
Fe	711	5.5	3991	1.0	1027	3.7
Hg	0.006	16.7	0.01	8.7	0.004	25.0
Mn	221	5.4	614	1.1	57.3	3.5
Mo	0.6	6.6	3.9	1.2	< 0.25	
Ni	4.5	4.6	24.4	3.2	14.3	8.8
Pb	3.2	5.8	3.2	5.3	1.2	1.2
V	16.8	5.6	131	0.4	25.1	3.9
Zn	107	1.1	211	2.2	11.6	10.2

Al = aluminum; As = arsenic; Cd = cadmium; Co = cobalt; Cr = chromium; Cu = copper; F = fluorine; Fe = iron; Hg = mercury; Mn = manganese; Mo = molybdenum; Ni = nickel; Pb = lead; V = vanadium and Zn = zinc.
n = 6 (replicates/sample).

32.53% for the simple linear equation (Tibia-breaking strength = 1.10141 + 1.16426 x phosphorus consumption from phosphate extracted from swine wastewater; Tibia-breaking strength = - 0.899610 + 3.57865 x phosphorus consumption from dicalcium phosphate; R^2 = 0.94) and 31.38% for the multiple linear equation (Tibia-breaking-strength = 2.84357 - 1.96469 x phosphorus consumption from basal diet + 3.12149 phosphorus consumption from phosphate extracted from swine wastewater + 9.94736 x phosphorus consumption from dicalcium phosphate; R^2 = 0.95).

Despite the low availability, phosphate from swine wastewater shows environmental gains, when compared to commercial phosphates. Also, it is worth to mention that it is free from chemical elements and microbiological contamination (*E. coli* and *Salmonella* sp. were absence, table 4). These

Table 3 - Tibia-breaking strength and phosphorus consumption.

Treatment, g/kg	Tibia-breaking strength, kgf	----------------Phosphorus consumption in dry matter (g)-------------			
		Total	Basal	PESW[1]	Dicalcium phosphate
Control, 0	1.72	0.60	0.60	---	---
PESW, 0.5	2.05	0.70	0.61	0.08	---
PESW, 1.0	2.03	0.85	0.67	0.18	---
PESW, 1.5	2.40	1.08	0.77	0.31	---
PESW, 2.0	2.54	1.28	0.83	0.45	---
Dicalcium phosphate, 0.5	2.25	0.92	0.81	---	0.11
Dicalcium phosphate, 1.0	3.65	1.40	1.10	---	0.30
Dicalcium phosphate, 1.5	5.54	1.95	1.39	---	0.56
Dicalcium phosphate, 2.0	8.25	2.38	1.54	---	0.84

[1]Phosphate extracted from swine wastewater.
n = 270.

Table 4 - Microbiological assay of pathogens.

Microorganisms	PESW[1]	Dicalcium phosphate	Hydrated lime
Salmonella sp.	Absence in 25 g[2]	Absence in 25 g[2]	Absence in 25 g[2]
E. coli	< 1.10^2/g[2]	< 1.10^2/g[2]	< 1.10^2/g[2]

[1]Phosphate extracted from swine wastewater.
[2]Bacterial counts below the minimum established by the Brazilian Health Regulatory Agency (Anvisa) implies in safe ingredients.

benefits are important, given that poultry production without bacteria is essential, because when birds are infected, they may cause future harm by spreading contamination to other animal species, besides contamination might negatively affect poultry performance.

CONCLUSION

Phosphate extracted from swine wastewater was negative for inorganic contaminants and pathogens (*E. coli* and *Salmonella* sp.). The average availability of phosphate extracted from swine wastewater is 31%.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

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

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The project was submitted and approved by the Comissão de Ética no Uso de Animais (CEUA - Embrapa) under protocol number 012/2011 and is therefore in accordance with the obligations of Law 11.794/2008 (BRASIL, 2008), with Decree 6,899/2009, and with the norms established by the Conselho Nacional de Controle da Experimentação Animal (CONCEA).

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