

Revista Brasileira de Zootecnia © 2009 Sociedade Brasileira de Zootecnia ISSN 1516-3598 (impresso) ISSN 1806-9290 (*on-line*) www.sbz.org.br

R. Bras. Zootec., v.38, n.1, p.90-98, 2009

Phosphorus availability of rock phosphates as compared with feed-grade phosphates for swine¹

Luiz Waldemar de Oliveira Souza², Anibal de Sant'Anna Moretti³, Fernanda Marcussi Tucci⁴, Nedilse Helena de Souza⁵, Paulo Ademar Martins Leal⁶, Nelson Haruo Anzai³

¹ Pesquisa financiada pela FAPESP protocolos nº 96/12815-1 e 97/12050-8.

² Faculdades Integradas de Ourinhos, FEMM.

³ Faculdade de Medicina Veterinária e Zootecnia, USP.

⁴ Secretaria de Defesa Agropecuária, MAPA.

⁵ Estação Experimental de Lages, EPAGRI.

⁶ Faculdade de Engenharia Agricola, UNICAMP.

ABSTRACT - One hundred ninety two swine were used in a trial to assess the relative bioavailability of phosphorus (RBP) in six phosphate sources. Phosphates were three feed grade phosphates (FP), two made in Brasil, and one USA made, and three rock phosphate samples (RP) originated from two mines sites in Brasil, and one mine site in Israel. Levels of calcium, phosphorus and fluorine in RP were 29, 12 and 1.7% (RP source 1), 33, 14 and 1.4% (RP source 2), and 30, 14 and 3.6% (RP source 3), respectively. Pigs were fed a corn-soybean meal basal diet (18% CP, 0.95% Lys, 0.75% Ca, 0.37% P) or the basal diet with 0.15% P from a standard purified grade calcium phosphate (SP), or with 0.15% P from experimental FP or RP. Each diet was fed to six pen replicates of four pigs per pen for 35 days (14.4 to 39.9 kg). Weight gain (WG), feed/gain (FG), plasma P (PP), bone ash (BA), and breaking strength of metacarpals and metatarsals (BS-MM) and femurs (BS-F) were improved by phosphorus addition. However, performance and bone parameters were depressed by RP, as compared to FP dietary supplementation. WG, BA, BS-MM and BS-F were regressed to P added, and slope-ratios were calculated to assess RBP in the FP and RP sources. The average bioavailability of P in the FP and RP sources, relative to SP, were 89 and 49% (WG), 112 and 49% (BA), 78 and 28% (BS-MM), and 101 and 52% (BS-F), respectively. Low animal performance and bone strength related to toxicity should be expected if rock phosphates are used to feed pigs.

Key Words: available phosphorus, bone strength, performance, piglets

Disponibilidade de fósforo em fosfatos de rocha em comparação à de fosfatos bicálcicos para suínos

RESUMO - Cento e noventa e dois leitões foram usados em um experimento para avaliar a biodisponibilidade relativa do fósforo (RBP) em seis fontes fosfáticas. As fontes foram três fosfatos de uso em nutrição (FP), dois fabricados no Brasil e um nos Estados Unidos, e três amostras de fosfatos de rocha (RP), originados de duas minas brasileiras e uma mina situada em Israel. Os níveis de cálcio (Ca), fósforo (P) e flúor (F) nos fosfatos de rocha foram 29, 12 e 1,7% (fonte RP 1), 33, 14 e 1,4% (fonte RP 2) e 30, 14 e 3,6% (fonte RP 3), respectivamente. Os suínos foram alimentados com uma dieta basal de milho e farelo de soja (18% PB, 0,95% Lis, 0,75% Ca, 0,37% P) ou dieta basal contendo 0,15% P a partir de um fosfato bicálcico purificado padrão (SP), ou com 0,15% P a partir das fontes experimentais FP ou RP. Cada dieta foi fornecida a seis baias (replicatas) com quatro leitões durante 35 dias (14,4 a 39,9 kg). O ganho de peso (WG), a conversão alimentar (FG), o P plasmático (PP), as cinzas ósseas (BA) e a resistência óssea à quebra de metacarpos e metatarsos (BS-MM) e fêmures (BS-F) melhoraram com a adição de fósforo às dietas. Contudo, o desempenho e os parâmetros ósseos pioraram com o uso dos fosfatos de rocha, em comparação à suplementação dietética de FP. A análise de regressão dos dados de WG, BA, BS-MM e BS-F em relação ao P adicional foi realizada e foram calculadas *slope-ratios* para avaliar a RBP nas fontes de FP e RP. A biodisponibilidade média do P nas fontes FP e RP, relativas ao SP (com valor atribuído de 100), foram 89 e 49% (WG), 112 e 49% (BA), 78 e 28% (BS-MM), e 101 e 52% (BS-F), respectivamente. Baixos desempenho e resistência óssea relacionados à toxicidade devem ser esperados se fosfatos de rocha forem utilizados na alimentação de suínos.

Palavras-chave: desempenho, disponibilidade de fósforo, leitões, resistência óssea

Received July 2, 2007 and accepted May 8, 2008. Corresponding author: lwsouza@dr.com

Introduction

Animal diets based on corn and soybean meal are very deficient in available phosphorus (P) to the point that supplementation is necessary using sources of high P availability. Dicalcium phosphate is commonly used as a source of supplemental P. Commercial dicalcium phosphate is a mixture of varying amounts of monocalcium and dicalcium phosphates, phosphoric acid, calcium carbonate, and impurities (Lima et al., 1995). Some animal production technicians point out that rock phosphates is an alternative dietary P source in animal nutrition due to their very low price compared to feed grade phosphates. It is well known that rock phosphates are not intended to be used in animal diets for not being submitted to manufacturing procedures to guarantee the adequate purity degree for feeding foodproducing animals, and to their very low P bioavailability. In addition, high levels of certain mineral elements can be toxic to the animal.

Digestibility methods are not adequate for feed availability determination. They only give apparent digestibility of the total P in the diet (Cromwell, 1992) and do not allow to assess the P digestibility in individual ingredients. Slope ratio has been chosen to assess the P availability by many researchers. In general, the P availability is calculated as a relative biological value compared with a standard phosphate source (purified grade) and is expressed simultaneously from several biological response criteria, such as animal performance and bone characteristics. Performance and bone characteristics have shown to be an easy and efficient method to study the available P in feedstuffs.

In attempt to determine the P bioavailability in feed grade phosphates (FP) and rock phosphates (RP), and to verify if the use of such alternative P sources could be used in animal nutrition, an experiment with growing pigs fed with FP or RP as a supplemental P source was carried out. The aim of this study was to evaluate three FP and three RP, using a purified-grade calcium phosphate as the reference standard on performance, blood and bone characteristics of growing pigs.

Material and Methods

Three rock phosphates were tested along with three feed-grade phosphates. The reference standard phosphate (SP) was a purified-grade dibasic dehydrate calcium phosphate (CaHPO₄.2H₂O) produced in Brazil. Feed-grade dicalcium phosphates FP-1 and FP-2 were products from

the Brazilian phosphate industry and FP-3 (monodicalcium phosphate) was from U.S. RP-1 and RP-2 were obtained from Brazilian mines (Araxá and Tapira), and RP-3 was from Arad mine site in Israel (Table 1).

Standard analytical methods recommended by AOAC (1995) were used. All phosphates were analyzed for moisture, insoluble residue, loss on ignition, and pH. Mineral analysis included the essential Ca, P, Mg, Na, K (macroelements), Co, Cu, Fe, Mn, Mo, S, Se, Zn (microelements), and the potentially toxic elements Al, F, As, B, Ba, Bi, Cd, Cr, Hg, Ni, Pb, Sb, Sn, Ti, V, W, and the radioactive U.

Analysis were performed at the IMC-Agrico laboratory, which employed a segmented-flux analytical system for Ca and P determinations and an ion selective electrode for fluorine analysis. Atomic absorption (Perkin-Elmer model 5100, Perkin-Elmer, Norwalk, CT, 06859) was used for Al, Fe, and Mg. Trace-elements were analyzed by plasma spectrometry (Perkin-Elmer Soiax Elan 500) and moisture determinations were performed using a vacuum oven.

pH values were obtained from 5 g of phosphate samples in 250 mL solutions (water pH = 7.0) using a potentiometer. Moisture was determined at 80°C, basically to quantify the hygroscopicity of the products, since water of crystallization losses may occur with temperatures as low as 109°C. Loss on ignition was determined at 1,000°C in dry samples and represents losses in water of crystallization, carbon dioxide from carbonates and volatile mineral elements such as As, Hg and halogens. Insoluble residue in HCl followed by HNO3 basically quantifies the silica content.

All X-ray diffraction assays were performed at the "Laboratório de Caracterização Tecnológica" (LCT) laboratory using methodology described by Lima et al., 1995, for identification of the chemical species present in the samples. Analytical procedures employed an X-ray diffractometer PHILLIPS-PW1880 (PHILLIPS, 7602 EA,

Table 1 - Analytical specifications and composition of phosphates¹

P source	e Origin	Ca (%) maximum	P (%) minimum	F (%) maximum
SP ²	Dicalcium, Brazil	24.0	18.0	_
FP ³ -1	Dicalcium, Brazil	24.0	18.0	0.18
FP-2	Dicalcium, Brazil	23.0	19.0	0.19
FP-3	Dicalcium, USA	24.0	18.5	0.19
RP ⁴ -1	Araxá, Brazil	29.0	12.0	1.7
RP-2	Tapira, Brazil	33.0	14.0	1.4
RP-3	Arad, Israel	30.0	14.0	3.6

¹ Minimum P and maximum Ca and F, as guaranteed by manufacturers (FP), or typical P, Ca, and F levels as suggested by the manufacturers (SP and RP).

 2 SP = standard phosphate.

³ FP = feed-grade phosphate.

 4 RP = rock phosphate.

Almelo, Holland) with a PW1710 controller and PC-APD (automated powder diffraction) software.

Ingredients from the diets were analyzed according to AOAC (1995) methods to determine dry matter, crude protein, ether extract, ash, Ca and P. A corn and soybean meal-based diet (Table 2) was formulated to contain adequate levels of all nutrients, except for P, in order to meet the swine requirements (NRC, 1998), and contained 0.37% of P. The supplemented diets were formulated by using appropriate levels of each one of the seven P sources to provide 0.15% of additional phosphorus (making 0.52% of total P). The calcium content of the diets was kept constant (0.75%) by varying the limestone and kaolin levels added. Calcium and phosphorus analyses in the diets were in agreement with the calculated values.

One hundred and ninety-two crossbred pigs with initial average weight of 14.3 kg, were distributed as a complete randomized block design into eight treatments [(4 P sources (without P, SP, FP[3], RP[3] x 2 genders [barrows e gilts)] with six replicates blocks (pens) of four pigs per pen (two barrows and two gilts) each, for 35 days (14.4 to 39.9 kg). Pigs were housed in forty-eight 2.25 x 0.73 m partially slotted heated-floor pens, in groups of 2 barrows and 2 gilts per pen. The appropriate experimental diet and water were provided *ad libitum* throughout the entire 35-d experimental period. Weight gain, feed intake, and feed gain for each pen were recorded at 7, 14, 21, 28 and 35 days.

T 11 0	a	C . 1		
Table 2 -	Composition	of the	experimental	diets
1 4010 2	composition	or the	experimental	areas

At the end of the experiment, one barrow and one guilt from each pen were randomly selected for blood sampling by ear vein puncture, and one barrow and one guilt were also randomly selected from each pen and slaughtered (electrical stunning followed by exsanguination). Metacarpal, metatarsal and femur were excised, and were cleaned from muscles and connective tissues, and tested for bone strength. To measure the bone strength, each metacarpal and metatarsal was held by two supports spaced 2.5 cm apart (5 cm for femurs) and force was applied to the midpoint of the bone by a probe attached to a 50 kg load cell (500 kg for femurs), with a crosshead speed of 50 mm/min (Ottawa Texture Measuring Instrument)1. The breaking strength was recorded in kilograms by pen deflection through a recorder. The same metacarpal and metatarsal samples were subsequently extracted with diethyl ether for 8 h. Combined metacarpal and metatarsal bone samples from each replicate were ashed at 600°C overnight for ash content determination.

The plasma was separated by blood centrifugation for $10 \min at 2,000 \ge g$ and saved for plasma calcium, phosphorus, and *alkaline phosphatase* (ALP) determinations. Plasma samples were processed by an autoanalyzer Technicon (model RA-100)2. Plasma characteristics were determined according to methods described by Connerty and Briggs (1966) for calcium, Gomorry (1942) and Raabe (1955) for phosphorus, and Bowers and McComb (1966) for ALP.

Item		Source of additional P									
Ingredient	None (basal diet)	Standard phosphate	Feed-grade phosphate			Rock phosphate					
	BD	SP	FP-1	FP-2	FP-3	RP-1	RP-2	RP-3			
Ground yellow corn	70.98	70.98	70.98	70.98	70.98	70.98	70.98	70.98			
Soybean meal (48.5% CP)	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00			
Iodized salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50			
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08			
L-lysine chloride	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04			
Vitamin and mineral premix ¹	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20			
Test mineral supplement ²	0.0	2.20	2.20	2.20	2.20	2.20	2.20	2.20			
Limestone	0.0	1.32	1.32	1.50	1.47	1.03	1.0	1.16			
Kaolin	0.0	0.05	0.05	0.09	0.00	0.00	0.03	0.00			
Phosphate	0.0	0.83	0.83	0.79	0.81	1.25	1.07	1.07			
Calculated analysis											
ME, kcal.kg ⁻¹	3,290	3,290	3,290	3,290	3,290	3,290	3,290	3,290			
CP, %	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00			
Lysine, %	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95			
Methionine + Cystine, %	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60			
Ca, %	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75			
Total P, % diet	0.37	0.52	0.52	0.52	0.52	0.52	0.52	0.52			
Nonphytate P, %	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13			

¹ Supplied per kilogram of diet : vit A, 11,250 IU; cholecalciferol, 2,250 IU; vit E, 22.5 mg; menadione, 2 mg; thiamine, 1.75 mg; riboflavin, 5 mg; piridoxine, 1.75 mg; vit B₁₂, 0.0225 mg; niacin, 37.5 mg; calcium pantothenate, 20 mg; folic acid, 0.5 mg; biotin, 0.5 mg; BHT, 125 mg; Se, 0.25 mg; Fe, 100 mg; Cu, 15 mg; Zn, 125 mg; Mn, 87.5 mg; I,1.25 mg.

² Test mineral supplement contains variable amounts of limestone, kaolin and tested phosphates to provide 0.15% of added P.

Statistical analysis was performed according to a complete randomized block design. The 35-d weight gain, feed intake, feed:gain ratio, plasma Ca, P and ALP, as well as bone ash percentage, and bone breaking strength data were analyzed using analysis of variance procedures of SAS software (SAS, 1990). The analysis of data adopted the statistic model represented by $Y_{ij} = m + B_i + S_j + e_{ij}$, where Y_{ii} is the observed value of the variable response of the cage; m is the estimated mean; B_i is the block effect; S_i is the source effect; e_{ii} is the experimental error. Orthogonal comparisons were used to test the effects of the various phosphate groups, by partitioning the 7 degrees of freedom for the treatment effect. The relative bioavailability of phosphorus (RBP) for each test phosphate was determined by the "three point assay" as described by Ammerman et al. (1995). Body weight gain, bone ash percentage, and bone breaking strength values were regressed on phosphorus added to basal diet within each phosphorus source and the slope of the regression line for each test source was divided by the slope of the standard phosphate (100% RBP). Values obtained for the control group were used as a common intercept in regression equations to estimate the Pavailability.

Results and Discussion

Analytical values obtained for the three FP sources were all in compliance with the manufacturer's guarantee levels. The most predominant chemical substances present in all commercial FP were calcium phosphates and calcium carbonates (Table 3). For the reference standard, purifiedgrade dibasic dehydrated calcium phosphate, dibasic dehydrated calcium phosphate seems to be the most predominant product found.

Among the feed-grade samples, monocalcium phosphate was found only in the U.S. di-monocalcium phosphate (FP-3). The anhydrous salt of dicalcium phosphate was identified as the most predominant substance present in FP-1, FP-2, and FP-3. Calcitic limestone was found in large proportions in all three commercial dicalcium phosphate products and may be the result of excess calcium carbonate added to neutralize the phosphoric acid during the industrial processing.

These results are in agreement with previous publication from Lima et al. (1995, 1999), which reported X-ray diffraction evaluations for two pure and ten commercial dicalcium phosphates and found that monocalcium phosphate was present in only some commercial products. According to these reports, the most predominant chemical species present in all commercial dicalcium phosphates were calcium carbonate and anhydrous dicalcium phosphate. These results are consistent with the pH values obtained (Table 4), showing the lowest value for FP-3 (pH=4.3), when compared to SP (pH=7.0), and FP-1 (pH=7.8), and FP-2 (pH=7.2).

Considering the rock phosphates, the most predominant chemical substance present was fluorohapatite, followed by smaller amounts of barium sulfate and iron oxide (RP-1), calcium carbonates (RP-2 and RP-3), and titanium oxide (RP-2).

In routine feed formulation for swine and poultry, phosphate supplements are largely used at levels not to exceed the 2% limit in conventional diets. Considering the swine requirements for minerals according to the NRC (1998), Cu, Mg, Mn, Se and Zn levels present in feed phosphates are inconsequential.

The comparison between the maximum tolerable level of several mineral elements for domestic animals, as suggested by the NRC (2005) and values observed in this

Table 3 - X-ray diffraction-presence of chemical species of phosphates

P source	Chemical specie					
	Major	Minor				
SP ¹	CaHPO ₄ .2H ₂ O					
FP ² -1	CaHPO ₄ ²	CaCO ₃				
FP-2	CaHPO ₄	CaCO ₃ KMg ₃ Si ₃				
	MgHPO ₄ .3H ₂ O	$AlO_{10}(F,OH)_2$				
FP-3	$Ca(H_2PO_4)_2.\tilde{H_2}O$	CaCO ₃				
	CaHPO ₄	-				
RP ³ -1	$Ca_5(PO_4)_3F$	BaSO ₄ FeO(OH)				
RP-2	$Ca_5(PO_4)_3$	CaMg(CO ₃) ₂ TiO ₂				
	FCaCO ₃					
RP-3	$Ca_5(PO_4)_3F$	CaCO ₃				

¹ SP = standard phosphate.

 2 FP = feed-grade phosphate.

 3 RP = rock phosphate.

Table 4 - Moisture, insoluble residue, loss on ignition, and pH of phosphates

P source	Moisture %	Insoluble residue %	Loss on ignition %	pН
SP ¹	0.24	0.08	24.30	7.0
FP ² -1	1.58	0.31	14.93	7.8
FP-2	0.91	1.36	17.76	7.2
FP-3	2.01	1.28	18.97	4.3
RP ³ -1	0.16	4.66	1.18	7.1
RP-2	0.13	3.90	3.95	9.4
RP-3	1.75	2.52	8.09	8.8

 1 SP = standard phosphate.

 2 FP = feed-grade phosphate.

 3 RP = rock phosphate.

				P source			
Item	SP^1	FP ² -1	FP-2	FP-3	RP ³ -1	RP-2	RP-3
Essential	mineral elemen	ts					
				%			_
Ca	24.30	25.71	22.37	21.12	34.36	36.29	37.34
Р	18.06	19.73	18.32	18.55	15.51	15.03	14.51
Mg	0.03	1.12	2.52	0.64	0.11	1.03	0.12
Na	0.12	0.14	0.14	0.14	0.28	0.18	0.44
K	0.01	0.05	0.06	0.01	0.02	0.04	0.06
				ppn	n		
Co	<1	2	13	3	8	5	1
Cu	<1	5	18	1	8	1	21
Fe	ND^4	2,300	13,100	10,600	19,500	6,200	600
Mn	5	293	796	290	666	282	11
Mo	1	1	1	10	3	1	7
S	600	1,000	9,900	10,100	2,300	200	6,800
Se	<5	<5	<5	<5	<5	<5	<5
Zn	<1	104	21	57	238	3	401
Potential	y harmful miner	al elements					
				% .			-
Al	ND	0.34	0.22	0.74	0.11	0.03	0.82
F	0,02	0.14	0.13	0.13	2.58	1.45	4.04
				ppn	n		
As	<1	3	3	9	11	10	8

34

4

< 0.1

3

70

< 0.5

16

2

2

ND

36

120

<5

160

4

9,243

< 0.1

1

14

< 0.5

34

21

< 1

ND

20

44

<5

12

Table 5 -	Essential and	l potentially harmfu	l mineral element	levels of phosphates
-----------	---------------	----------------------	-------------------	----------------------

¹ SP = standard phosphate.

4

11

< 0.1

< 1

1

< 0.5

1

<1

< 1

ND

ND

2

<5

4

 2 FP = feed-grade phosphate.

 3 RP = rock phosphate. 4 ND = not detectable.

В

Ba

Bi

Cd

Cr

Hg

Ni

Pb

Sb

Sn

Тi

V

W

U

study (Table 5) indicates no toxicity risks for the use of any of the feed phosphates studied in normal diets for swine.

10

13

< 0.1

< 1

9

< 0.5

7

< 1

< 1

ND

86

54

<5

12

6

532

< 0.1

< 1

13

<0.5

11

3

<1

ND

82

43

<5

15

Previous publications from Lima et al. (1995, 1999) reported analytical values for 26 to 31 mineral elements in three pure dicalcium phosphates, and in fourteen commercial dicalcium phosphate samples. The comparison between the average results for three dicalcium phosphate samples obtained in this study and the levels reported by Lima et al. (1995, 1999) showed comparable values for most mineral elements analyzed.

Nutritionally essential element concentrations in common macromineral supplements have been reported by NRC (2005). Fe, Cu, Mg, Mn, and Zn levels are generally compared to those obtained in this study.

In contrast to the safety demonstrated for feed-grade phosphates in supplementing animal diets, this does not seem to be true for the rock phosphates tested. Rock phosphates may represent considerable risk of toxicity for use in animal diets, considering their F and Ba levels. According to recommendations from the AAFCO (1973), phosphate sources for use in animal feeds should not contain more than 1 part of F to 100 parts of P, and according to NRC (2005), the maximum tolerable level for swine in phosphate sources, used at a dietary level of 2%, is 1,000 ppm for Ba. Rock from Araxa (RP-1) was found to contain 1,510% excess F (2.58 vs 0.16%), and 1,330% excess Ba (14,270 vs 1,000 ppm). The F level of Tapira phosphate (RP-2) exceeded the tolerance limit by 870% (1.45 vs 0.15 %). The

5

446

< 0.1

< 1

1

< 0.5

5

3

< 1

ND

152

94

<5

6

32

186

< 0.1

15

171

< 0.5

41

1

2

ND

16

187

<5

139

F level of the Arad sample (RP-3) was found to exceed by 2,790% the tolerance limit (4.04 vs 0.15%).

These results are in line with the findings of Lima et al. (1999), who found for three FP and seven RP samples, that the mineral concentrations were safe for all FP when compared to NRC or European standards. However, the RP levels exceeded the tolerance limits for F, Ba, As, Pb and Cd, and were particularly high when compared to FP, for the radioactive Th. Tapira rock was high in F and As, Araxá rock was high in F, Ba, As, Pb and Th, rock from Patos de Minas was high in F, two rock samples from Catalão, Copebras and Goiasfértil, were high in F, Ba, and As, Arad and North Carolina rocks were high in F, As and Cd.

Higher level of dietary P improved the performance parameters, regardless the P source (P<0.01), when compared to the control diet (Table 6). Considering an average value for all 7 supplemented diets, compared with the control dietfeed pigs, the performance was improved (P<0.01) by 22.4% for BW gain (522 vs 460 g/d), 8.4% for FI (1,360 vs 1,255 g/d), and 18% for FG (2.34 vs 2.85). These findings are in agreement with Cromwell et al. (1970), which observed improvements in BW gain, FI and FG of pigs in response to increase dietary P. Similar results were recorded for broilers by Fernandes et al. (1999), who studied four feed-grade, and four agricultural-grade phosphates, as well as one standard purified grade P source, and reported that the performance was improved by 31% for BW, 34% for BW gain, 7% for FI, and 20% for FG, in response to increasing dietary P (0.08 or 0.16% P added to a 0.48% P basal diet).

The toxicity of feeding rock phosphates to pigs was clearly shown by the evaluation of the performance results.

The performance was strongly depressed (P<0.01) by supplementing diets with rock phosphates (RP-1, 2 or 3) when compared to feeding the feed-grade products. Calculating an average value for the 3 diets supplemented with RP-1, 2 or 3 when compared to the FP-1, 2 or 3, the performance was depressed (P<0.01) by 18.5% for BW gain (522.3 vs 641.0 g/d), 9% for FI (1,276 vs 1,402 g/d), and 13% for FG (2.49 vs 2.20). These results were in disagreement with Plumlee et al. (1958), who found similar performance results for swine fed either with dicalcium phosphate or with soft rock phosphate in a 90-day feeding trial. However, the results of this study are in agreement with Potter (1988), who observed a poor performance for turkeys fed with Curaçao rock phosphate, and with the report of Barbosa et al. (1992), who recorded a reduction of 11.6% in the BW gain, and a depression of 5% in FG for swine fed with diets supplemented with rock phosphate originated from the Brazilian mine of Patos de Minas.

Similar results were recorded for broilers by Fernandes et al. (1999), who observed a decrease in performance by 11% for BW, 12% for BW gain, and 14% for FI when diets were supplemented with agricultural-grade phosphates, when compared to feed-grade phosphates. The results of this study were also in agreement with Garzillo et al. (1997), who reported that the performance was depressed (P<0.01) by supplementing diets with RP (7 samples), when compared to FP (3 samples) supplementation. Body weight was decreased by 7% (679.9 vs 729.2 g), BW gain by 7% (638.0 vs 687.1 g) and FI by 7% (1,003 vs 1,074 g).

Higher levels of dietary phosphorus increased the plasma phosphorus concentration (P<0.01). The average value of all supplemented diets was 32.9% higher than the basal diet (9.36 vs 6.28 mg/dL, respectively). Previous experiments have demonstrated that higher dietary P levels increase the plasma phosphorus concentrations (Plumlee et al., 1958; Mahan, 1982). However, the plasma calcium levels decreased as the plasma phosphorus levels increased (Mahan, 1982), in response to the dietary phosphorus addition. Maxson & Mahan (1983) also reported similar response of plasma phosphorus and calcium levels, with an inverse relationship between both minerals. Despite this relationship, it was observed in the present study that both level and source of phosphorus did not influence the calcium levels. Based on these results, it could be concluded that the plasma calcium concentration was a non-sensitive approach to assess the relative phosphorus bioavailability.

Alkaline phosphatase measurements showed a decrease of about 10.5% in the plasma concentrations when basal diet had 0.15% of additional phosphorus (481.5 vs 435.9 IU/L); however, the contrast between basal diet and supplemented diets was not significant. The average value for dicalcium phosphates (347 IU/L) was 57.3% lower (P<0.0001) than that observed for rock phosphates (545 IU/L). While FP decreased the plasma levels (346 IU/L), RP promoted the highest levels in all classes of phosphorus source (545 IU/L), which raised the average of the supplemented diets. Thus, the difference between basal diet (482 IU/L) and average for all supplemented diets (436 IU/L) was not significant. This fact was confirmed by the difference (P<0.01) between dicalcium phosphate and rock phosphate. Reduction by 2.6% in plasma P was not significant when rock was compared to dicalcium phosphate. On the other hand, Boyd et al. (1983) observed ALP activity declining along increasing dietary P levels, until the diet reached levels close to NRC (1998) minimum requirements. Although the present results showed differences between feed grade and rock phosphates only for ALP, the influence

of rock phosphate in the P concentrations was noted by Plumlee et al. (1958), who verified that supplementation by dicalcium phosphate increased (P<0.01) the P levels more than Curaçao rock phosphate.

Lower levels of dietary P (basal diet) caused lower metacarpal and metatarsal, and femur breaking strength (P<0.01), and BA (P<0.01). When swine received additional P, the average of BS-MM was 18.7 kg, BS-F was 157 kg, and BA was 29.26%. Swine fed with basal diet had 14.3 kg, 81 kg, and 25.86%, respectively. These results showed an improvement of 23.8% (metacarpal/metatarsal) and 48.3% (femur) in BS and 11.6% in the mineral content of bones, when diets had additional P. The increasing in BS-MM was according to Cromwell et al. (1972), who observed increasing from 125.9 to 141.1 kg of metacarpal breaking strength when dietary phosphorus increased in 0.15%. The average BS-F was reduced (P<0.01) almost to half (from 157 to 81 kg) when animals did not receive P supplementation. Also, the average BA was lower (P < 0.01) for non-supplemented pigs (25.86%) when compared to supplemented animals (29.26%).

Elaborated phosphorus sources improved (P>0.01) metacarpal and metatarsal, and BS-F. The average BS for swine supplemented with dicalcium sources was 18.6%

higher than rock sources (20.1 vs 16.4 kg). The same behavior was observed by Plumlee et al. (1958) who reported higher BS-F (P<0.05) in response to feed-grade phosphate supplementation than that obtained for soft phosphates. Gomes et al. (1992) observed improved (P<0.05) bone ash with FP in comparison to rock phosphate. Also, higher metacarpal and femur breaking strength values were observed (P<0.01). Both, BA and BS reduction were probably result of less available P on diets supplemented with RP. For BS-F, the difference was 26.2% (177 vs 131 kg, respectively). Bone ash was improved in 10.8% (30.6 vs 27.3%, respectively). All bone measurements showed difference (P<0.01) between feed-grade and rock phosphates. The same behavior was reported by Fernandes et al. (1999), who observed that diets supplemented with agricultural phosphates caused a decrease (P<0.01) in bone ash and breaking strength of broilers, when compared to FP.

Coefficients of RBP determination were expressed as percentage of the dibasic dehydrate standard calcium phosphate (purified-grade), for which P was considered to be 100% available (Table 7). All FP samples were more available than RP samples, considering any of the studied parameters.

 Table 6 - Performance, blood plasma and bone parameters of pigs fed with experimental phosphates (means, mean squares and orthogonal contrasts)

Phosphorus source		Pe	erform	ance		Blood plasma			Bo	ne measuren	nents
		′gain g/d)	FI (g/d)	FG	Plasm (mg/10		Plasma Ca (mg/100 mL)	ALP Units/L	BS-MM (kg)	BS-F (kg)	BA (%)
None	2	60	1,25	5 2.85	6.2	28	14.83	482	14.3	81.3	25.86
SP	6	62	1,48	7 2.29	9.4	40	11.75	376	21.7	176.3	31.08
FP-1	6	521	1,34	5 2.18	10.	29	11.11	328	18.6	183.2	33.68
FP-2	6	54	1,46	5 2.28	8.9	95	13.09	335	21.5	176.5	27.93
FP-3	6	648	1,40	2 2.20	9.	17	13.11	377	20.1	172.3	30.21
RP-1	4	48	1,15	0 2.63	9.0	06	11.37	600	15.0	126.3	26.76
RP-2	4	577	1,32	8 2.32	9.2	28	13.63	531	18.1	128.7	25.30
RP-3	5	542	1,27	6 2.52	9.3	34	11.82	505	15.9	137.3	29.84
Mean	5	576	1,34	7 2.40	8.9	97	12.59	442	18.2	147.8	28.83
SD	11	22.7	173.	5 0.42	1.4	46	3.14	114.2	3.28	37.7	3.16
ANOVA					N	Aean squa	res				
Sources of P	(7 df)	44,35	2**	71,737**	0.3523*	8.0916	** 9.9146	61,222**	49.68**	7,581**	49.55**
None vs supplemented	(1 df)	92,90	0**	58,118	1.3822**	49.6420	** 34.3424	10,926	103.83**	30,248**	60.60**
SP vs others	(1 df)	33,30	4*	113,115**	0.0201	0.0164	1.8617	25,100*	62.63**	2,552**	23.20**
FP vs RP	(1 df)	126,6	17**	143,515**	0.7253*	0.5208	0.2467	355,017**	124.73**	19,507**	98.37**
FP-1, -2 vs FP-3	(1 df)	380		380	0.0261	0.8130	4.0468	8,525	0.01	225	1.40
FP-1 vs FP-2	(1 df)	3,300		43,080	0.0271	5.3734	** 11.7414	147	25.55*	133	99.02**
RP-1, -2 vs RP-3	(1 df)	3,520		48,547	0.0093	0.1190	1.8405	14,762	1.69	387	57.86**
RP-1 vs RP-2	(1 df)	50,44		95,408*	0.2760	0.1564		14,077	29.36**	16	6.42

Duration of the feeding period: 28 d for performance, and 35 d for blood plasma and bone parameters.

None = no supplementation; SP = Standard Ca phosphate dibasic dehydrate (purified grade); FP = Feed-grade phosphate; RP = Rock phosphate.

*P<0.05; **P<0.01.

P source	r^2	Regression equation	SD	RBP ²
		BW gain		
SP ³	0.4818	Y = 460 + 1,347x	46.85	100.0
FP ⁴ -1	0.4548	Y = 460 + 1,073x	39.42	79.7
FP-2	0.4619	Y = 460 + 1,294x	46.86	96.0
FP-3	0.5957	Y = 460 + 1,249x	34.51	92.6
RP ⁵ -1	0.0038	Y = 460 - 83x	45.02	nd ⁶
RP-2	0.3186	Y = 460 + 781x	38.32	57.9
RP-3	0.1950	Y = 460 + 547x	37.26	40.6
		—— Bone strength, metacarpal-metatar		
SP ²	0.7782	Y = 14.3 + 49.6x	0.888	100.0
FP ³ -1	0.7025	Y = 14.3 + 29.0x	0.633	58.5
FP-2	0.8643	Y = 14.3 + 48.4x	0.644	97.7
FP-3	0.7349	Y = 14.3 + 38.8x	0.781	78.2
RP ⁴ -1	0.0657	Y = 14.3 + 4.9x	0.624	9.9
RP-2	0.8029	Y = 14.3 + 25.8x	0.429	52.0
RP-3	0.4225	Y = 14.3 + 11.0x	0.432	22.2
		Bone strength, femur -		
SP	0.9614	Y = 81.3 + 633x	4.26	100.0
FP-1	0.9632	Y = 81.3 + 679x	4.45	107.4
FP-2	0.9222	Y = 81.3 + 634x	6.18	100.0
FP-3	0.9125	Y = 81.3 + 607x	6.30	95.8
RP-1	0.7156	Y = 81.3 + 300x	6.34	47.4
RP-2	0.8393	Y = 81.3 + 316x	4.63	49.5
RP-3	0.8486	Y = 81.3 + 373x	5.29	58.9
		Bone ash	·	
SP	0.6970	Y = 25.9 + 34.8x	0.769	100.0
FP-1	0.8678	Y = 25.9 + 52.1x	0.682	146.3
FP-2	0.2439	Y = 25.9 + 13.8x	0.816	74.6
FP-3	0.5933	Y = 25.9 + 29.0x	0.806	115.2
RP-1	0.0926	Y = 25.9 + 6.0x	0.633	63.4
RP-2	0.0328	Y = 25.9 - 3.7x	0.678	34.9
RP-3	0.5300	Y = 25.9 + 26.5x	0.837	48.2

 Table 7 - Coefficients (r²) and regression equations for P sources using BW gain, bone ash and bone strength as criteria for P availability assessment, standard deviation and relative bioavailability of phosphorus¹

¹ Y = dependent variable (BW gain, bone ash, bone strength); a = intercept; b = slope; x = percentage supplemental P.

² Relative bioavailability of P.

³ SP = dibasic dehydrate standard CA phosphate (purified grade).

⁴ FP = feed-grade phosphate.

⁵ RP = rock phosphate.

⁶ Not determined.

Average RBP for BW gain was 89.4% and 49.2% for FP and RP, respectively. Among the several sources, the highest value for bioavailability was observed for SP. The RP-1 source resulted in negative intercept, being not possible to calculate the availability. This fact must be due to the lack of quality and excess of impurities, resulting in very low animal performance, when compared to FP sources. Gomes et al. (1992) observed 98.6% of RBP for RP-2 in relation to dicalcium phosphate.

Mean RBP values calculated through BS-MM for FP and RP were 78.1 and 28.0%, respectively. Once again, the lowest availability value was for RP-3 (9.9% BRP).

The RBP values observed for FP and RP were 101.1% and 51.9%, respectively, when considering BS-F.

The relative bioavailability of phosphorus of FP and RP relative to BA were 112 and 48.8%, respectively.

Conclusions

The analytical values obtained for the three feed-grade phosphate sources studied indicate that no mineral elements was found at toxic levels for use in animal diets. Analytical evaluations of the three rock phosphates studied revealed that these products could be toxic to animals. All feed-grade phosphates used in this trial resulted in better performance when compared to the use of rock phophates. Rock phosphates promoted lower bone strength and less bone ash when compared to feed-grade phosphates. The relative bioavailability of phosphorus from feed-grade phosphates was considered high, while rock phosphates showed poor phosphorus availability, which means lower animal production, higher levels of supplementation and more phosphorus releasing to the environment. Since most studies conducted to assess the bone characteristics revealed better breaking strength values and ash content for feedgrade phosphates, the results show that rock phosphate should not be used as source of supplementary phosphorus.

Acknowledgments

On behalf of his students, colleagues and many friends throughout Brazil and abroad, this paper is dedicated to the life, accomplishments and memories of their respected counselor, teacher, researcher and friend, the late Professor, Doctor Felix R. de Lima. Special acknowledgements are due to Dr Virgil W. Hays, former professor at the University of Kentucky and Dr Gary L. Cromwell, researcher at the University of Kentucky, for their cooperation with students of the Departamento de Nutrição e Produção Animal of FMVZ-USP.

The authors are grateful to IMC-Agrico, Mundelein, IL, 60060, Ralston Purina, Paulinia, SP, Brazil in laboratory analysis. The assistance of Mitsui S.A., São Paulo, SP, 01311-940, Brazil, and Fosfértil, Uberaba, MG, 38102-970, Brazil in obtaining some phosphate source samples is acknowledged.

Literature Cited

- AMMERMAN, C.B.; BAKER, D.H.; LEWIS, A.J. Bioavailability of nutrients for animals. San Diego: Academic Press, 1995. 441p. ASSOCIATION OF AMERICAN FEED CONTROL OFFICIALS -
- AAFCO. **Official publication**. Charleston: 1973. p.62. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC.
- Official methods of analysis. 16.ed. Arlington: 1995. 1025p.
- BARBOSA, H.P.; FIALHO, E.T.; GOMES, P.C. Efeitos de fontes alternativas de fósforo no desempenho e características do osso de suínos em crescimento e terminação. **Boletim da Indústria Animal**, v.49, p.63-72, 1992.
- BOYD, R.D.; HALL, D.; WU, J.F. Plasma alkaline phosphatase as a criterion for determining biological availability of phosphorus for swine. Journal of Animal Science, v.57, n.2, p.396-401, 1983.
- BOWERS JR., G.N.; McCOMB, R.B. A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. **Clinical Chemistry**, v.12, p.70-89, 1966.

- CONNERTY, H.V.; BRIGGS, A.R. Determination of serum calcium by means of orthocresolphtalein comnplexon. American Journal of Clinical Pathology, v.45, p.290-296, 1966.
- CROMWELL, G.L. The biological availability of phosphorus in feedstuffs for pigs. **Pig News and Information**, v.13, p.75-78, 1992.
- CROMWELL, G.L.; HAYS, V.W.; CHANEY, C.H. et al. Effects of dietary phosphorus and calcium level on performance, bone mineralization and carcass characteristics of swine. Journal of Animal Science, v.30, n.4, p.519-525, 1970.
- CROMWELL, G.L.; HAYS, V.W.; SCHERER, C.W. et al. Effects of dietary calcium and phosphorus on performance carcass, metacarpal and turbinate characteristics of swine. Journal of Animal Science, v.34, n.5, p.743-751, 1972.
- FERNANDES, J.I.M.; LIMA, F.R.; MENDONÇA JR., C.X. et al. Relative bioavailability of phosphorus in feed and agricultural phosphates for poultry. **Journal of Poultry Science**, v.78, p.1729-1736, 1999.
- GARZILLO, J.M.F.; LIMA, F.R.; DAGLI, M.L.Z. et al. Tibial histomorphometry data can explain differences in phosphorus availability in high fluorine rock phosphates. In: INTERNATIONAL CONGRESS OF THE WORLD VETERINARY POULTRY ASSOCIATION, 11., 1997, Budapest, Hungary. **Proceedings...** Budapest: 1997. p.348.
- GOMES, P.C.; LIMA, G.J.M.M.; BARBOSA, H.P. et al. Disponibilidade de fósforo nos fosfatos de Tapira e Fosforindus e na farinha de ossos para suínos. Revista da Sociedade Brazileira de Zootecnia, v.21, n.1, p.83-89, 1992.
- GOMORRY, G. A modification of colorimetric phosphorus determination for use with the photoelectric colorimeter. Journal of Laboratory Clinical Medicine, v.27, p.955-960, 1942.
- LIMA, F.R.; FERNANDES, J.I.M.; OLIVEIRA, E. et al. Laboratory evaluations of feed-grade and agricultural-grade phosphates. Journal of Poultry Science, v.78, p.1717-1728, 1999.
- LIMA, F.R.; MENDONÇA JR., C.X.; ALVAREZ, J.C. et al. Chemical and physical evaluations of commercial dicalcium phosphates as sources of biologically available phosphorus for broiler chicks. Journal of Poultry Science, v.74, p.1659-1670, 1995.
- MAHAN, D.C. Dietary calcium and phosphorus levels for weanling swine. Journal of Animal Science, v.54, n.3, p.559-564, 1982.
- MAXSON, P.F.; MAHAN, D.C. Dietary calcium and phosphorus levels for growing swine from 18 to 57 kilograms body weight. Journal of Animal Science, v.56, n.5, p.1124-1134, 1983.
- NATIONAL RESEARCH COUNCIL NRC. Nutrient requirement of swine. 10.ed. Washington, D.C.: National Academy of Sciences, 1998. 189p.
- NATIONAL RESEARCH COUNCIL NRC. Mineral tolerance of animals. 2.ed. Washington, D.C.: National Academy of Sciences, 2005. 510p.
- PLUMLEE, M.P.; JORDAN, C.E.; KENNINGTON, M.H. et al. Availability of the phosphorus from various phosphate materials for swine. Journal of Animal Science, v.17, p.73-88, 1958.
- POTTER, L.M. Bioavailability of phosphorus from various phosphates based on body weight and toe ash measurements. Journal of Poultry Science, v.67, p.96-102, 1988.
- RAABE, S. Die beteutung routinemasseger phosphatase bestimmungen in klinischen labors. Recueil des Travaux Chimiques des Pays-Bas e de la Belgique, v.76, p.681, 1955.
- STATISTICAL ANALYSIS SYSTEM SAS. **SAS/STAT®** user's guide: statistics. Release 6.04 Edition. Cary: 1990.