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Phosphorus utilization in broilers fed with diets supplemented with different feed ingredients

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ABSTRACT: A reliable determination of endogenous phosphorus (P) excretion is required to measure P utilization in chickens accurately. The objective of this study was to investigate phosphorus (P) retention in broilers fed diets formulated with different feed ingredients. Sixty-four 15-d old broiler chicks were fed diets in which part of the dietary P was provided from dicalcium phosphate, maize, barley or soybean. Level of supplementation of each ingredient was calculated to provide two levels of total P (4.5 or 5.0 g kg^{-1} feed). Birds received a single injection of 3 MBq of ^{32}P to determine endogenous P excretion using the isotope dilution technique. Four days after injection, blood and excreta were collected for analysis of inorganic and radioactive P. There were no differences among diets in total ($p = 0.37$) or endogenous ($p = 0.65$) P excretion or in P retention ($p = 0.37$) regardless of the supplemental feed material used in each diet. Daily P retention was increased ($p = 0.004$) as P intake increased, but the proportion of P ingested that was retained was not affected ($p = 0.23$) by the level of dietary P. The use of an isotopic tracer allows for accurate estimation of endogenous P in excreta, ranging from 0.24 to 0.42 mg P g^{-1} dry matter intake. The retention of P in growing chickens was not changed when 10-20 % of total P was provided by maize, barley or soybean.

Keywords: chicken, endogenous, isotope dilution, phosphorus retention

Introduction

Phosphorus utilization by poultry depends on the form of dietary P, as P in phytate form in plant feedstuffs is poorly available (Humer et al., 2015). The environmental fate of P in poultry manure also depends on P form (Leytem et al., 2008a). When P is provided in the form of phytate, dietary allowances are usually in excess to ensure that the animal requirements are matched (Li et al., 2016). In these situations, large amounts of phytate P are excreted and can pose an environmental risk to soil and aquatic compartments when manure is applied to land (Knowlton et al., 2004).

Most P in cereal grains is present in phytate form (~70 % of total P). The strong chelating potential of phytate often compromises the use of other minerals such as calcium (Ca), zinc, magnesium and copper (Singh, 2008) thus affecting the involvement of P in most metabolic processes and bone development. There is evidence that phytate may also affect negatively protein and energy utilization in poultry (Ravindran et al., 2000; Scott et al., 2001; Shirley and Edwards, 2003; Selle et al., 2012). In contrast, an increase in P utilization by degradation of phytate adding a microbial phytase to the diet has been consistently shown to enhance growth performance (Simons et al., 1990; Cabahug et al., 1999).

The phytate source can affect P digestibility due to the presence of intrinsic phytase activity in feedstuffs (Kornegay, 2001; Humer et al., 2015). Phytase activities are low in legume seeds, intermediate in cereals (except oats) and high in cereal by-products (Viveros et al., 2000; Steiner et al., 2007). The presence of phytase in some cereal grains such as wheat and barley can be beneficial to phytate utilization by poultry (Kornegay, 2001; Singh, 2008). Phytate from different sources may differ in terms of solubility and susceptibility to hydrolysis (Dersjant-Li et al., 2015). Due to variability in the P properties in different feedstuffs that can affect its availability, it is of paramount significance to determine the P availability of different P sources commonly used in poultry diets. However, estimation of true P retention requires accurate determination of endogenous P excretion. Given the methodological difficulties of discriminating the endogenous P in excreta, few studies have quantified this fraction. An alternative to determine endogenous P is the isotope dilution technique. Information on P availability offers reassurance to nutritionists and poultry producers interested in optimizing P utilization in broilers when formulating diets (Shastak and Rodehutscord, 2013). In this context, the objective of this study is to assess P retention from three conventional feeds (maize, barley, and soybean) by determining endogenous P excretion in broilers fed diets varying in total P content.

Materials and Methods

The experiment was conducted in Pirassununga, Sao Paulo, Brazil (latitude 21°57′33″ S; longitude 47°28′1″ W; altitude 627 m).

Animals and experiment design

Sixty-four male broiler chicks (Cobb line) were randomly assigned to eight treatments with four replicates each (two chicks per replicate) in a factorial arrangement of 2 × 4 (two levels of P supplementation and four sources of P). The intended concentrations of total P in the diets were 4.5 or 5.0 g total P kg^{-1} feed and the sources of additional P (over a basal level common to all the diets) were dicalcium phosphate, maize, barley and soybean.

Diets

A semi-purified basal diet containing maize starch and soybean meal was formulated to meet the nutritional requirements of broiler chickens from one to 21 d of age according to the National Research Council (NRC, 1994), except for P, which was below the recommended allowances for this bird category. The basal diet was formulated to contain 4.0 g of total P kg^{-1} feed and supplemented diets contained approximately 4.5 and 5.0 g of total P kg^{-1} feed at low and high P diets, respectively. Both rates of addition of total P (0.5 and 1.0 g kg^{-1} feed over the total P provided by the basal diet) were achieved using each of the four sources (dicalcium phosphate, maize, barley or soybean meal) in substitution of maize starch to formulate the eight diets shown in Table 1. Chicks were fed the corresponding experimental diet daily *ad libitum* for 15 d before receiving a radioactive P injection. Water was always available. Dietary Ca levels were provided at a 2:1 ratio with P by adjusting the quantities of limestone in the diet. All diets were formulated to be isocaloric (2900 kcal metabolizable energy kg^{-1}) by varying the incorporation of soya oil into the diet, at the expense of maize starch. DL-Methionine was added at different rates to achieve similar concentrations of methionine + cysteine in all diets.

Experiment

On the first day of the experiment, 64 chicks were placed in 32 wire cages (1.0 m × 0.40 m, 2 birds per cage) equipped with electrical heating, feeder (0.70 m) and two water nipples. During the adaptation period (first 15 d), the chicks were fed with the corresponding experimental diet. On day 16, each bird received an intra-peritoneal injection of radioactive $Na_2H^{32}PO_4$ (São Paulo, Brazil) as a single dose of 3 MBq of ^{32}P in 0.5 mL of a sterile isotonic solution. On day 20, 5 mL of blood was collected from the left wing using vacutainer tubes. Total excreta from each cage were collected daily from day 16 to 20 of the experiment to determine DM and total P and estimate DM digestibility and true P retention. Excreta collected on day 20 were used for the analysis of ^{32}P to determine endogenous P excretion. The experimental procedures were approved by the Bioethics Commission of FMVZ/USP (Protocol number CEEA001/2004).

Chemical analysis

Feed samples and feed refusals were analyzed for DM, crude protein (CP) and Ca following recommendations of the Association of Official Analytical Chemists (1995). Inorganic P was determined in feed, excreta

Table 1 – Diets composition.

Source of P supplementation	Dicalcium phosphate		Maize		Barley		Soybean	
Total P (g kg^{-1} diet)	4.5	5.0	4.5	5.0	4.5	5.0	4.5	5.0
Ingredients (%)								
Soybean meal	48.6	48.6	48.6	48.6	48.6	48.6	56.6	63.6
Maize starch	42.0	41.0	23.0	4.0	26.0	10.0	32.0	22.0
Dextrose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soya oil	1.08	1.35	2.08	3.36	3.09	5.37	3.16	5.52
Maize			18.0	35.0				
Barley					14.0	28.0		
Limestone (38 % Ca)	1.53	1.61	1.69	1.94	1.69	1.94	1.64	1.85
Dicalcium phosphate[1]	0.74	1.02	0.47	0.47	0.47	0.47	0.47	0.47
DL-methionine	0.20	0.20	0.14	0.07	0.13	0.05	0.10	0.00
Choline chloride	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin-Mineral supplement[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Chemical composition								
Crude Protein (%)	22.1	22.0	23.5	24.9	23.6	25.2	25.4	28.8
Calcium (%)	0.91	1.01	0.90	1.00	0.90	1.02	0.89	0.99
Phosphorus (%)	0.44	0.49	0.44	0.48	0.43	0.48	0.44	0.48
Lysine (%)[3]	1.36	1.36	1.40	1.44	1.41	1.47	1.58	1.77
Methionine (%)[3]	0.51	0.51	0.48	0.44	0.47	0.41	0.46	0.41
Methionine + cysteine (%)[3]	0.84	0.84	0.84	0.83	0.83	0.81	0.85	0.84

[1]18 % P and 24 % Ca; [2]The vitamin-mineral supplement provides, per kg of diet: 10,000 UI vitamin A, 2.000 UI vitamin D, 25 mg vitamin E, 3 mg vitamin K, 2 mg thiamine, 6 mg riboflavin, 30 mg nicotinic acid, 12 mg pantothenic acid, 4 mg pyridoxine, 12 µg vitamin B_{12}, 1 mg folic acid, 0.08 mg biotin, 40 mg iron, 12 mg copper, 120 mg zinc, 100 mg manganese, 2.5 mg iodine and 0.75 mg cobalt; [3]Amino acid contents are calculated from values tabulated for the ingredients of each diet.

and blood by colorimetry using the vanadate molybdate reagent (Fiske and Subbarow, 1925; Sarruge and Haag, 1974) and a method adapted from the Association of Official Analytical Chemists (1995), described in detail by Dias et al. (2013). Excreta were collected daily and frozen until analysis. After the experimental period, excreta were thawed, dried at 65 °C and weighed. Thereafter, they were milled through a 1 mm sieve, weighed again and dried in an oven at 105 °C to determine DM. Excreta were then incinerated in a muffle at 550 °C for 4 h, and solubilized in HCl to obtain a mineral solution for subsequent analysis of P using the aforementioned method.

Radioactive ^{32}P was analyzed in the material injected and in the blood and excreta collected 96 h after injection. Excreta of each cage were ground in a mortar and later homogenized. Then, a sample (1 g) of excreta was placed in a porcelain crucible for determination of DM (after drying at 100 °C) and ash (after incinerating at 500 °C). Ashes were digested with 10 mL of H_2SO_4 (18 N). The digested material was placed in a scintillation vial, completing the volume to 20 mL with distilled water. Radioactivity of ^{32}P was measured in a Packard Liquid Scintillation Spectrometer (model 2450B) using Cerenkov radiation.

Calculations

Endogenous losses of P were calculated according to Lofgreen and Kleiber (1953):

$$\text{Endogenous loss of P (g d}^{-1}) = (SA_{excreta}/SA_{plasma}) \times \text{total P in excreta (g d}^{-1})$$

where: $SA_{excreta}$ is ^{32}P enrichment in excreta and SA_{plasma} is ^{32}P enrichment in plasma, both in atom percent excess. We used the definitions and calculations proposed by Shastak and Rodehutscord (2013) and the World Poultry Science Association (2013) to assess P utilization in broilers based on determinations of P intake and P voided in excreta. Using the values of endogenous P loss in excreta, P retention from the diet was calculated as:

True P retention (g d^{-1}) = P intake – total P in excreta + endogenous P

True P retention coefficient (as proportion of dietary total P) = P retention/P intake

Statistical analysis

Data were analyzed as a completely randomized design and ANOVA was conducted using the GLM procedure of SAS (Statistical Analysis System, version 9.1). A factorial arrangement of treatments was used with the following factors: source of additional P (dicalcium phosphate, maize, barley or soybean meal) and dietary P level (4.5 vs. 5.0 g total P kg^{-1} feed). The experimental unit was cage (with two chicks per cage), with four replicates (cages) per treatment.

The correlations between parameters expressed as the Pearson product-moment correlation coefficient were determined using PROC CORR of SAS.

Results

The mean values of total P intake and excretion, endogenous P excretion, true P retention and true P retention coefficient are shown in Table 2.

There was no significant interaction between the effects of P source and dietary P level in any response variable ($p > 0.05$). The analysis of the main effects showed that P sources did not affect ($p > 0.05$) DM intake and digestibility, total P excretion, endogenous P excretion, P retention and retainable P. As expected, there were significant differences between levels of dietary total P in P retention. DM intake was also increased ($p > 0.05$) with greater dietary P content. Phosphorus intake was the same for all sources of P ($p > 0.05$); however, as part of the treatments, there were two different levels of P intake (Table 2). P intake was positively correlated with P retention ($r = 0.88$; $p < 0.0001$); however, it was not significantly correlated with total P excretion ($r = 0.19$; $p = 0.27$), endogenous P excretion ($r = 0.10$; $p = 0.57$) or retainable P ($r = 0.30$; $p = 0.09$).

Table 2 – Effects of source and level of total phosphorus (P) supplementation on P intake, excretion and utilization in broiler chickens.

	Source of supplemental P (S)				SEM	Dietary P level (L)		SEM	*p*-values		
	Dicalcium phosphate	Maize	Barley	Soya		4.5	5.0		S	L	S × L
	g total P kg^{-1} feed										
DM intake (g d^{-1})	56.6	53.9	58.5	50.5	3.12	51.3	58.4	2.21	0.318	0.031	0.283
DM digestibility (g absorbed g^{-1} ingested)	0.772	0.800	0.754	0.730	0.0249	0.754	0.774	0.0176	0.268	0.438	0.197
P intake (g d^{-1})	0.270	0.256	0.279	0.241	0.0147	0.231	0.293	0.0104	0.314	< 0.001	0.334
P excretion (g d^{-1})	0.083	0.063	0.075	0.075	0.0080	0.067	0.081	0.0056	0.374	0.097	0.247
P plasma (mg dL^{-1})	2.97	2.11	2.63	2.06	0.294	2.07	2.82	0.207	0.111	0.018	0.303
Apparent retainable P (g retained g^{-1} ingested)	0.689	0.743	0.722	0.690	0.0363	0.702	0.720	0.0256	0.665	0.611	0.589
Endogenous P excreted in excreta (g d^{-1})	0.024	0.022	0.019	0.012	0.0072	0.014	0.025	0.0051	0.651	0.159	0.810
True P retention (g d^{-1})	0.214	0.214	0.222	0.179	0.0181	0.178	0.236	0.0128	0.368	0.004	0.486
True P retention coefficient (g retained g^{-1} ingested)	0.775	0.821	0.794	0.742	0.0350	0.762	0.804	0.0248	0.452	0.233	0.411

SEM = standard error of the mean.

Discussion

In the present study, the P sources were dicalcium phosphate, maize, barley and soybean, which are widely used in poultry nutrition and show different P phytate contents and phytase activities (Ravindran et al., 1994; Singh, 2008) that may influence P utilization. In addition, P utilization by non-ruminants can be affected by dietary P level (Viveros et al., 2002). In all the diets used in this study, 61-69 % of total P was provided by soybean, and 18-20 % of total P by dicalcium phosphate. The rest (11 % of total P in the low P diets, and 20 % of total P in the high P diets) was provided by different feed sources, namely additional dicalcium phosphate, maize, barley or soybean.

Endogenous P excretion was determined using the isotope dilution technique, which allows discriminating between excretion of undigested dietary P and excretion of endogenous P (Lofgreen and Kleiber, 1953). Although this technique yields reliable values of endogenous excretion, it is not routinely used, as it requires specific equipment and procedures to deal appropriately with radioactive material. These difficulties limit the number of animals and replicates that could be used in this type of study. Therefore, the available literature on endogenous P determined through this technique is scarce. Al-Masri (1995) investigated P utilization in male broiler chicks aged 14-29 d using the dilution technique and reported mean values of 0.030 and 0.031 g d^{-1} of endogenous P excretion for chicks fed Ca:P ratios of 2:1 and 2.5:1, respectively. These values are in agreement with the overall mean value of 0.023 g d^{-1} found in our study where the Ca:P ratio in feed was kept close to 2:1 and the chicks were around 2 weeks of age.

The endogenous P values observed in our study are lower than 0.08 g d^{-1} observed by Dilger and Adeola (2006) for growing male broiler chicks fed conventional and low phytate grains. Liu et al. (2013) also obtained higher estimates of endogenous P (726-803 mg kg^{-1} DM intake) with diets differing in Ca:P ratio (ratios from 0.8:1 up to 2:1). In both studies, the regression of excreta output against dietary intake (both in mg P kg^{-1} DM intake) was used to estimate the endogenous loss and, possibly, the different methodology used explains in part the discrepancy with the present study (where an isotopic tracer technique was used).

In our study, endogenous excretion was not affected by P source or P level in the diet. Thus, despite the different techniques used to determine endogenous P (isotope dilution technique versus regression analysis), our results agree with Dilger and Adeola (2006), which suggest that endogenous P excretion is not influenced by dietary phytate or total P content of the diet.

An adequate rate of P absorption is essential for the health and development of growing chicks due to the demand for P for bone development. As mentioned earlier, P absorption in birds can be affected by the type of P in the feed ingredient (Singh, 2008). It is well known that P in phytate form is less available for absorption and further utilization by poultry than inorganic P. In addition, availability of phytate can vary according to its solubility and the presence of intrinsic plant phytase, which can improve phytate utilization (Singh, 2008). Thus, it is important to have a better understanding of the differences in P availability among the main sources of P used in poultry diets.

Phosphorus retention values are in close agreement with the value 0.16 g d^{-1} found by Al-Masri (1995) for the same category of bird fed diets having a similar Ca:P ratio. However, P retention did not differ among the different P sources, indicating that any differences in phytate levels or presence/absence of intrinsic phytase related to these sources did not affect P utilization. Mutucumarana et al. (2014) observed that true ileal P digestibility was different in broiler diets formulated with different feed ingredients (wheat, sorghum, soybean meal, and corn dried distillers grains with solubles). The experimental conditions of our study differed substantially from those of the experiment reported by Mutucumarana et al. (2014). Greater P retention and P concentration in plasma were observed in birds fed a higher level of P in the diet. This increased P retention is attributed to greater P intake, not to enhanced feed P utilization in the digestive tract. Xue et al. (2016) have shown that dietary protein can affect P utilization in broiler chickens, in particular when growth is limited by protein deficiency in the diet, reducing P retention. As the dietary P was changed by adjusting the level of inclusion of the different feed ingredients, there were small differences among diets in the protein and lysine contents (Table 1), mainly between low and high P diets with the same feed ingredient. Although it is possible that nutrients other than P (e.g., protein) may influence P utilization, possibly, it is not the explanation for the differences between low and high P diets in P retention, because all diets were formulated to provide protein and amino acids to support maximum growth rate in broilers (NRC, 1994).

Phosphorus retention was not affected by an interaction between P sources and different P levels. Ballam et al. (1984) observed that hydrolysis of phytate P was reduced as dietary Ca and non-phytate P (mineral P from dicalcium phosphate) were increased. Leske and Coon (1999) showed that when phytase was not added to the feeds, there were no significant differences between maize, barley and soybean in the amounts of phytate P hydrolyzed or in total P retention in broilers fed diets with these feeds as the only P source. In our study, the increased supply of mineral P from dicalcium phosphate in the basal diets had no apparent effect on P retention compared to the other feed sources. The increased supply of non-phytate P from dicalcium phosphate could be offset by a reduced hydrolysis of phytate P in the basal diet, resulting in a lack of significant differences between diets in P reten-

tion. Mohammed et al. (1991) observed that different dietary P levels did not affect phytate utilization whereas lower dietary Ca level elevated phytate digestibility. Leytem et al. (2008a) concluded that phytate P was less digestible when large amounts of Ca and P are added to poultry diets regardless of the type of grain fed or level of intrinsic phytase in the diet. Leytem et al. (2008b) observed no differences between cereals (maize, wheat or barley) in phytate digestibility, indicating that intrinsic phytase in these grains may have a minor effect on phytate degradation. Similar to our study, more than 60 % of total P was provided by soybean meal in all diets and all diets contained at least 4.7 g dicalcium phosphate kg^{-1} feed. Under these conditions, it is plausible that differences between diets in non-phytate P were not enough to cause differences in P retention.

Although there is sufficient evidence that the addition of phytase to diets for growing chicks improves P utilization (Perney et al., 1993; Sohail and Roland, 1999), there is little information on P availability in typical feeds used in poultry diets. This information could aid the producer to formulate diets to optimize P utilization by considering the availability of P from different feeds. Precise supply of dietary P should be adjusted to the specific requirement for available P, highlighting the need for information about availability of P from feed ingredients (Knowlton et al., 2004).

When 10-20 % of total P is provided by maize, barley or soybean included in diets containing soybean and dicalcium phosphate, P retention is not reduced in spite of a greater supply of phytate. Therefore, poultry producers and nutritionists are assured that with these feed ingredients at the levels of inclusion used in the current study (to provide 10-20 % of total P), P utilization is not negatively affected in growing broiler chicks. However, different levels of inclusion of the ingredients, or a different basal diet, or broilers at different growth stages, or animals of a different genotype, could have led to a different response. Therefore, further research is needed before any extrapolation to higher levels of inclusion can be made.

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Authors' Contributions

Conceptualization: López, S., Vitti, D.M.S.S., Abdalla, A.L., France, J. Data Acquisition: Borgatti, L.M.O., Abdalla, A.L. Data Analysis: Dias, R.S., López, S., Borgatti, L.M.O., Abdalla, A.L., France, J. Design of Methodology: Dias, R.S., López, S., Vitti, D.M.S.S., Abdalla, A.L., France, J. Writing and Editing: Dias, R.S., López, S., Kebreab, E., Appuhamy, J.A.D.R.N., France, J.

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