Comparison of retention and prececal digestibility measurements in evaluating mineral phosphorus sources in broilers[1,2]

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ABSTRACT The objectives of this study were to compare measurements of retention and prececal (pc) digestibility in evaluating mineral phosphorus (P) sources in 3- and 5-wk-old broilers. A corn-soybean meal-based basal diet was used (0.35% P on DM basis). Anhydrous monosodium phosphate (MSP_a) or anhydrous dibasic calcium phosphate (DCP_a) was supplemented to increment the P concentration by 0.08%, 0.16%, and 0.24%. Titanium dioxide was used as the indigestible marker. Two retention trials with excreta collection from d 16 to 20 and d 30 to 34 were conducted (n = 8 birds per diet). Another 8 pens of 10 birds from the same hatch were allocated to each diet on d 11 or 25 each to measure pc digestibility in both age periods. After 10 d of feeding, these birds were euthanized and the content of a defined section of the terminal ileum was obtained. Percentage P retention and pc digestibility for MSP_a and DCP_a were calculated by linear regression analysis. In 3-wk-old broilers, P retention for MSP_a was 70% and significantly higher ($P < 0.001$) than for DCP_a (29%). Values determined for pc digestibility at the same age were very similar (67% for MSP_a and 30% for DCP_a; $P < 0.001$). In 5-wk-old broilers, P retention was 63% (MSP_a) and 29% (DCP_a; $P < 0.001$) and pc digestibility was 54% (MSP_a) and 25% (DCP_a; $P = 0.002$). We concluded that both retention and pc digestibility can be used for evaluating mineral P sources in broilers based on a regression approach. In 3-wk-old broilers, results obtained with both approaches were the same. In 5-wk-old broilers, the ranking of the 2 P sources was also the same for both approaches. Values did not differ significantly between the 2 age periods, but further studies on the relevance of broilers' age in P evaluation are suggested.

Key words: phosphorus, retention, prececal digestibility, availability

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INTRODUCTION

Plant ingredients are the main basis for compound poultry feed. Phosphorus (P) in plant ingredients is largely present in the form of phytic acid and its salts (Eeckhout and de Paepe, 1994), and in this form only poorly available to poultry (NRC, 1994). Although more than half of the pig and poultry diets on a global scale contain an exogenous phytase for enhancing P digestibility (Selle et al., 2009), mineral phosphates are needed to supplement poultry diets.

Most of the P used in agriculture as fertilizer or feed phosphate is derived from phosphate rock, which is a nonrenewable resource, and current global reserves may be depleted in 50 to100 yr (Cordel et al., 2009). Future generations ultimately will face problems in obtaining enough to exist, and further research is needed to avert problems on the long-term (Abelson, 1999). Optimizing the supply of available P in poultry diets and consideration of differences in the availability of different P sources is one approach to address the problem. Knowledge about the availability of P from mineral sources is important also because they are expensive in use and excessive excretion of P by livestock may impair the environment. Extending and improving the database for P availability in feed ingredients, including variation within one ingredient, is therefore urgently needed (Rodehutscord et al., 2012). "Availability" in the context of this paper is a criterion that describes the potential of a P source. It is understood as that proportion of dietary total P that, at marginal level of P supply, can be utilized to cover the P requirements of an animal (Rodehutscord, 2009).

Different experimental techniques are in use to measure or estimate P availability (Rodehutscord, 2009). Quantitative measurement of P retention is one way. The experimental effort is high and balance cages are

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needed, but the measurements are precise and can be directly interpreted as P availability. Assays based on growth, bone, or blood criteria are able to provide only relative values of P availability. Therefore, the information obtained from a relative biological availability assay has limited value for a nutritionist formulating diets (Coon et al., 2002). For evaluation of feed proteins and amino acids, measurements of disappearance at the end of ileum are very common in broiler studies, because they exclude the effects of postileal fermentation (Ravindran et al., 1999). Such measurements of prececal (**pc**) digestibility may be an alternative to retention measurements in P evaluation. The contribution of regulatory P excretion in the urine can be excluded and for this reason, pc measurements are the preferred approach for P evaluation in laying hens (Van der Klis et al., 1997; Rodehutscord et al., 2002). It was shown in broilers that the response in P pc digestibility to increments in dietary P concentration is linear over a wider range of dietary P than the response in P retention (Rodehutscord et al., 2012). It is not clear, however, whether measurements based on retention and pc digestibility deliver the same results when different mineral P sources are compared for their availability in broilers.

Some studies have examined either retention or pc digestibility of P from mineral sources, mostly at one certain age of birds and often using semipurified or purified types of diets (Ketels and De Groote, 1988; Van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997; Leske and Coon, 2002; Wendt and Rodehutscord, 2004; Rodehutscord and Dieckmann, 2005). These studies have demonstrated that birds can utilize P from different mineral sources to a different extent. However, published data are difficult to compare, because it is not clear to what extent details of the methodological approach affected the results and possibly masked real differences between the P sources. One relevant factor can be the age of birds. Fonolla et al. (1981) found significantly lower Ca and P retention for maize meal-barley meal-soybean meal-based diets in 52-d compared with 21-d-old broilers. This can be taken as an indication for an age effect on P availability, but differences may have been caused by an excessive intake of P in the older birds.

Our objectives were therefore (1) to compare the availability of 2 mineral phosphates determined with a regression approach based on either P retention or pc digestibility, and (2) to compare the measurements between 3-wk- and 5-wk-old broilers. Two balance trials and 2 digestibility trials were conducted.

MATERIALS AND METHODS

Birds and Management

Unsexed broiler hatchlings (Ross 308) were obtained from a local hatchery (Brüterei Süd, Regenstauf, Germany) and randomly allocated, 11 per pen, to 112 floor pens (154 × 154 cm) bedded with pine shavings. The temperature in the animal house was gradually reduced from 35 to 27.5°C between d 1 and 7, from 27 to 24°C between d 8 and 14, and from 23.5 to 22°C between d 15 and 21, after which it remained constant. Water was always available from a nipple drinker and feed from a feeder trough. Before the trials started, chicks were provided free access to a broiler starter diet containing (per kg) 238 g of CP, 12.5 MJ of ME, 10.6 g of Ca, and 7.1 g of P [4.5 g nonphytin P (**nPP**)]. A total of 20 h of light was provided per day throughout the study. Mortality was recorded daily. The study was approved by the Animal Welfare Commissioner of the University in accordance with Animal Welfare Regulations.

Experimental Diets

The diets were formulated to meet or exceed the requirements of the Gesellschaft für Ernährungsphysiologie (1999) for all nutrients except for Ca and P. Main ingredients, such as corn, soybean meal, potato protein, and corn starch, were chosen to achieve a low P concentration and low intrinsic phytase activity. Concentrations of Ca and P were calculated to be 0.7% and 0.35% in the basal diet (**BD**, on DM basis; Table 1). Anhydrous dibasic calcium phosphate (**DCP_a**; Sigma-Aldrich Inc., St. Louis, MO) and anhydrous monosodium phosphate (**MSP_a**; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) were used as the 2 mineral P sources under test. Together with limestone, inclusions of DCP_a and MSP_a were varied to achieve the targeted concentrations in another 6 diets. These test diets were calculated to contain 0.08%, 0.16%, or 0.24% of supplemental P from each of the 2 mineral P sources. The P and Ca levels were adjusted by replacing cellulose and sand in the BD with respective levels of limestone, MSP_a, and DCP_a. The Ca concentration also was increased in a way that the ratio of Ca to P was similar in all diets. Titanium dioxide (Merck KGaA, Darmstadt, Germany) was included as an indigestible marker at a rate of 0.5%. Diets were pelleted without using steam through a pellet die with a hole diameter of 3 mm. Samples of diets were collected immediately before the beginning of the trials. Samples were ground through a 0.5-mm sieve of a grinding mill (Type ZM 1, Retsch GmbH, Haan, Germany) and stored at room temperature to await analyses. Analyzed concentrations of Ca and P confirmed the intended levels (Table 2).

Retention Trials

For the determination of P retention, 56 chicks with a BW of 240 ± 15 g (period 1) and 1,150 ± 50 g (period 2) were used. Different birds were used in period 2. Broilers were placed individually into balance cages (45 cm wide × 50 cm deep × 42.5 cm high) at d 11 (period 1) and 25 (period 2) of age. Birds were allocated at random to one of the 7 experimental diets (8 birds per diet). The balance cages were equipped with a trough

Table 1. Ingredient composition of the basal diet and analyzed concentrations

Ingredient composition (g/kg)	Amount
Corn	522
Soybean meal, solvent extracted, 51% CP	190
Potato protein, 75% CP	129
Corn starch	85
Soybean oil	20
D,L-Methionine	1.5
Mineral mix[1]	1.0
NaCl	1.0
Sodium bicarbonate	3.0
Vitamin mix[2]	1.5
Choline chloride	2.0
Titanium dioxide	5.0
Exchange mixture[3]	39.0
Analyzed concentrations (g/kg of DM)	
CP	255
Crude fat	60
Calcium	7.1
Phosphorus	3.5

[1]Mineral mix (Celita SG 1,GFT MBH, Memmingen, Germany) provided per kilogram of diet: Cu, 15 mg; I, 1.6 mg; Fe, 90 mg; Mn, 120 mg; Zn, 80 mg; Co, 0.6 mg; Se, 0.5 mg.

[2]Vitamin mix (Raiffeisen Kraftfutterwerke Süd GmbH, Würzburg, Germany) provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 2,250 IU; vitamin E, 22.5 mg; menadione, 1.8 mg; thiamine, 2.3 mg; riboflavin, 4.5 mg; niacin, 37.5 mg; Ca-D-pantothenate, 10.5 mg; pyridoxine, 4.5 mg; vitamin B_{12}, 23 µg; folic acid, 0.75 mg; biotin, 0.075 mg.

[3]Exchange mixture contained cellulose and sand (1:1) and limestone. In the 6 test diets, this mixture was partially replaced by anhydrous monosodium phosphate or anhydrous dibasic calcium phosphate, as detailed in the text. The limestone concentration was adjusted to maintain a Ca-to-P ratio in all diets of 2 to 1.

and a drinking water beaker at the front side. Excreta were collected from trays underneath the wire mesh floor of the pens. Each retention trial consisted of a 4-d period of adaptation to the respective experimental diet and a 5-d main balance period with restricted feeding and complete excreta collection. Daily feed allowance was 50 g/bird (period 1) and 100 g/bird (period 2), which was approximately 90% of the ad libitum intake measured with birds that received the BD during the 3 d before the start of the collection period. The slight restriction in the amount of feed should ensure that differences in P intake originated only from the supplemented mineral P sources. However, feed intake was incomplete for some individuals in period 1, and these refusals were recorded. Feed was offered in 2 meals per day at about 0800 and 1700 h. Excreta were collected for 5 d after the morning feeding from the tray underneath the pen. Excreta were bulk-sampled for each individual bird and stored at −20°C. Feathers were removed before each collection. Later, the excreta were defrosted, dried at 65°C in a convection oven (Heraeus UT 6760, Hanau, Germany), and ground as described for the feed above. Chicks were weighed individually on d 11 and d 20 (period 1) and on d 25 and 34 (period 2).

Digestibility Trials

For the determination of pc digestibility, 560 chicks at d 11 and 25 of age each were weighed individually and distributed among 56 floor pens with 10 birds per pen to minimize variation in mean BW among pens. Broilers were from the same flock as those of the retention trials. Each pen was randomly assigned to one of the 7 diets (8 replicated pens per diet). Feed and drinking water were freely available from one feeder and one nipple drinker per pen. Mortality was recorded on a daily basis. After 10 d of feeding the experimental diets, the birds were asphyxiated by CO_2 exposure. The abdominal cavity was immediately opened, the small intestine exposed, and the section between Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction dissected. The terminal two-thirds of this section were used for digesta sampling as suggested by Rodehutscord et al. (2012). The digesta was gently flushed out using double-distilled water, pooled from all birds of one pen, and frozen at −20°C. Later, the digesta samples were freeze-dried (Type 102000, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and ground as described for the feed above. Individual BW was recorded at d 11 and 21 (period 1) and at d 25 and 35 (period 2) of age, and the mean was calculated for each pen. Feed consumption was measured on a pen basis. Total BW gain and feed efficiency were calculated with adjustment for mortality.

Chemical Analyses and Calculations

Samples of feed, excreta, and ileal digesta were analyzed for DM (103°C), P, Ca, and Ti. To determine the

Table 2. Calculated and determined concentrations of total P and Ca in the experimental diets (g/kg of DM)

	Total P		Ca	
Item[1]	Calculated	Determined[2]	Calculated	Determined[2]
Basal diet	3.5	3.54 ± 0.03	7.1	7.13 ± 0.04
MSP_a (0.08% P)	4.3	4.27 ± 0.01	8.6	9.78 ± 0.12
MSP_a (0.16% P)	5.1	5.18 ± 0.09	10.2	10.78 ± 0.04
MSP_a (0.24% P)	5.9	5.93 ± 0.04	11.8	12.79 ± 0.19
DCP_a (0.08% P)	4.3	4.26 ± 0.05	8.6	8.16 ± 0.15
DCP_a (0.16% P)	5.1	5.06 ± 0.07	10.2	9.51 ± 0.16
DCP_a (0.24% P)	5.9	5.87 ± 0.07	11.8	11.59 ± 0.37

[1]MSP_a = anhydrous monosodium phosphate; DCP_a = anhydrous dibasic calcium phosphate.

[2]Mean and SD of 3 samples per diet (retention trial in period 1 and period 2, digestibility trial). Each analysis was run in duplicate.

Ca, P, and Ti concentrations, a modification of method 10.6.1 (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, 2006) as described in detail by Boguhn et al. (2009) was used for wet digestion. The Ti concentrations in the solutions were determined together with Ca and P concentrations using an inductively coupled plasma optical emission spectrometer (VISTA PRO, Varian Inc., Australia) and specific wavelengths for each element (Ca, 317.933; P, 213.618; and Ti, 334.941 nm). Feed samples were also analyzed for CP (method 4.1.1) and crude fat (method 5.1.1; VDLUFA, 2006).

The pc digestibility of P (y) was calculated for each diet on a pen basis according to the following equation:

$$y\ (\%) = 100 - 100 \times [(TiO_{2\ Diet} \times P_{\ Digesta})/(TiO_{2\ Digesta} \times P_{\ Diet})],$$

where $TiO_{2\ Diet}$ and $TiO_{2\ Digesta}$ equal analyzed concentrations of TiO_2 in the diet and digesta samples (g/kg), and $P_{\ Diet}$ and $P_{\ Digesta}$ equal analyzed concentrations of P in the diet and digesta samples (g/kg). The pc digestibility of Ca was calculated accordingly, using analyzed Ca concentrations. The retention of P and Ca was also calculated by using this equation, although feed intake and amount of excreta were quantified. This was done because we wanted to compare the 2 mineral P sources based on excreta and ileal digesta measurements and therefore intended to equalize the methodological approaches as much as possible.

The retention and pc digestibility of the 2 mineral P sources was obtained from the slope of linear regressions of the type $y = a + mx$, calculated between the level of added inorganic P (g/kg of feed DM) (x) and the retained or pc-digested amount of P (g/kg of feed DM) (y). The calculated slope (m) multiplied by 100 is the percentage retention or pc digestibility of the supplemented P source. By this regression, the response to the 2 P sources was separated from the P contained in the BD, and corrections for basal endogenous losses were not necessary. Calculations were made using data for the BD and the 3 levels of added P for each P source. Thus, the number of data sets used in regression analysis for each P source was 32. Regressions were calculated using GraphPad Prism 5.02 (Graph Pad Software Inc., San Diego, CA).

Statistical Data Analyses

The pen served as the experimental unit for statistical analyses in the digestibility trials and the individual bird was the experimental unit in the retention trials. Analysis of variance was performed to analyze data from each trial separately. Results expressed in percentage were arcsin-transformed before analysis. All data were analyzed using the procedure for linear mixed models (PROC MIXED) of the software package SAS for Windows (version 9.1.3, SAS Institute Inc., Cary, NC). The analysis was done in accordance with the model $y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + e_{ijk}$, where y_{ijk} is the parameter, μ is the overall mean, α_i is the phosphate type ($i = MSP_a$ and DCP_a), β_j is the level of phosphate, γ_{ij} is the interaction of phosphate type and level, and e_{ijk} is the error term. If necessary, heterogeneous variance was allowed for every combination of phosphate type and level.

RESULTS

Retention Trials

No significant effects of P level or P source or their interaction on feed intake and BW gain were detected in periods 1 and 2 (Table 3). In period 1, P retention for the BD was 54.5%. No significant interaction between P source and supplementation level was detected. The addition of 0.08%, 0.16%, and 0.24% P from MSP_a to the BD resulted in P retention values of 59.0%, 61.8%, and 60.3%, respectively, without a significant effect of the P level. For 0.08%, 0.16%, and 0.24% P DCP_a-supplemented diets, P retention values were 49.7%, 43.6%, and 45.1%, respectively, and again without a significant effect of the P level.

In period 2, P retention of the BD was 49.2%. There was no significant interaction between P source and P supplementation level. The addition of 0.08%, 0.16%, and 0.24% P from MSP_a to the BD resulted in P retention values of 55.2%, 56.3%, and 54.9%, respectively. For 0.08%, 0.16%, and 0.24% P DCP_a-supplemented diets, P retention values were 45.7%, 41.3%, and 41.8%, respectively. For both P sources, the effect of the P level was not significant.

Calcium retention was significantly improved in periods 1 ($P = 0.011$) and 2 ($P = 0.004$) when the P concentration was increased (Table 3). Calcium retention for the BD was 23.3% and 15.1% in periods 1 and 2, respectively.

The relationship between P intake and retained P in period 1 was linear (Figure 1, upper panel). Retention of P from MSP_a and DCP_a, determined by linear regression analysis, was 70% and 29%, respectively (Figure 1, upper panel). The difference between the slopes for MSP_a and DCP_a was significant ($P < 0.001$). In period 2, the response was similar to that in period 1 (Figure 2, upper panel). Retention of P from MSP_a and DCP_a was 63% and 29%, respectively. Again, the difference between the slopes for MSP_a and DCP_a was significant ($P < 0.001$).

Digestibility Trials

The BW gain, feed consumption, and feed per gain ratio were significantly improved by dietary P level, but not by P source, in 11- to 21-d-old birds ($P < 0.004$; Table 4). In 25- to 35-d-old birds, a significant interaction ($P = 0.043$) indicated that the effect of P level on BW gain was greater for MSP_a than for DCP_a. Mortal-

Table 3. The BW gain, feed intake, and retention of P and Ca of broilers at different ages fed the low-P basal diet or diets supplemented with 2 different sources and levels of mineral P in the retention trials (n = 8 broilers per treatment)[1]

		MSP_a			DCP_a				*P*-value (ANOVA)		
Period	BD	0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	Pooled SEM	P source	P level	Level × source
Period 1											
BW gain d 11–20, g	253	277	257	275	253	239	261	11.3	0.519	0.771	0.689
Feed intake d 15–19, g	228	243	230	248	231	218	238	9.5	0.630	0.177	0.880
P retention,[2] %	54.5	59.0	61.8	60.3	49.7	43.6	45.1	2.1	0.750	0.312	0.100
Ca retention,[2] %	23.3	37.0	40.9	41.0	24.0	21.8	34.4	2.6	0.062	0.011	0.245
Period 2											
BW gain d 25–34, g	424	468	457	454	463	429	439	14.6	0.852	0.208	0.719
Feed intake d 29–33, g	489	495	497	495	495	492	494	2.4	0.818	0.850	0.998
P retention,[2] %	49.2	55.2	56.3	54.9	45.7	41.3	41.8	1.3	0.856	0.110	0.190
Ca retention,[2] %	15.1	34.6	32.0	40.4	15.9	21.9	27.4	2.8	0.085	0.004	0.319

[1]BD: basal diet; MSP_a: anhydrous monosodium phosphate; DCP_a: anhydrous dibasic calcium phosphate.

[2]Retention of P and Ca is expressed in % of intake. Excreta were collected on d 16–20 and d 30–34, respectively, and retention was calculated based on the marker technique.

ity for the birds fed the BD or MSP_a (0.08%, 0.16%, and 0.24%) and DCP_a (0.08%, 0.16%, and 0.24%) supplemented diets were 3.8, 6.3, 1.3, 6.3, 1.3, 2.5, and 2.5 in period 1 and 0, 6.3, 2.5, 1.3, 0, 1.3, and 2.5% in period 2, respectively, and treatment effects were not significant.

In periods 1 and 2, the pc digestibility of P from the BD was 54.1% and 41.5%, respectively (Table 5). No significant interactions between P source and P supplementation level were detected. In both periods, the pc digestibility of P was not significantly affected by P level or P source. The pc digestibility of Ca from the

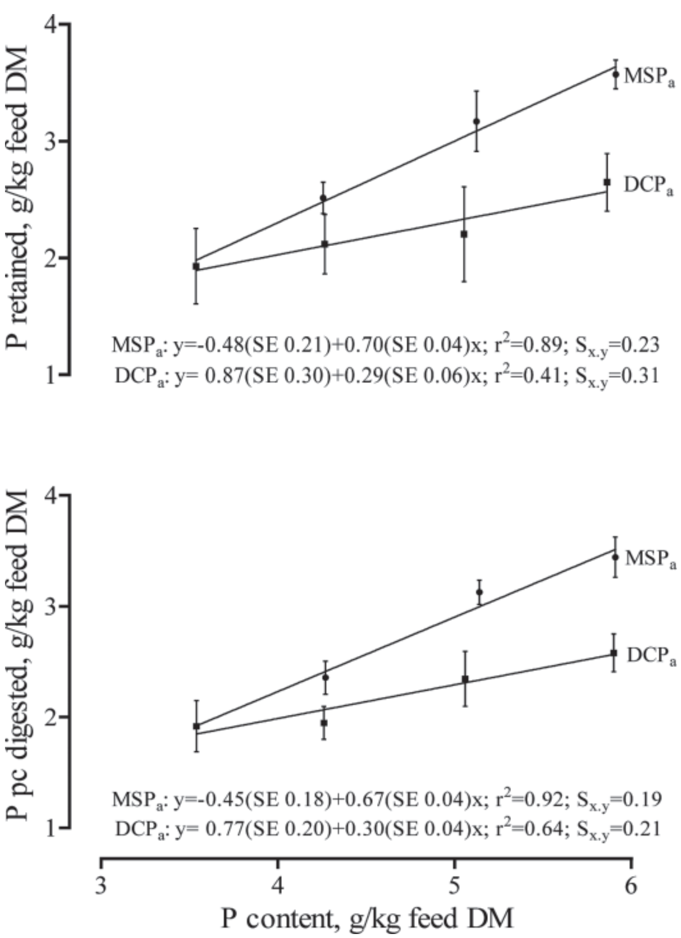

Figure 1. Phosphorus retention (upper panel) and P prececal (pc) digestibility (lower panel) depending on P content of diet in period 1 (mean and SD; n = 8 replicates per treatment). MSP_a = anhydrous monosodium phosphate; DCP_a = anhydrous dibasic calcium phosphate.

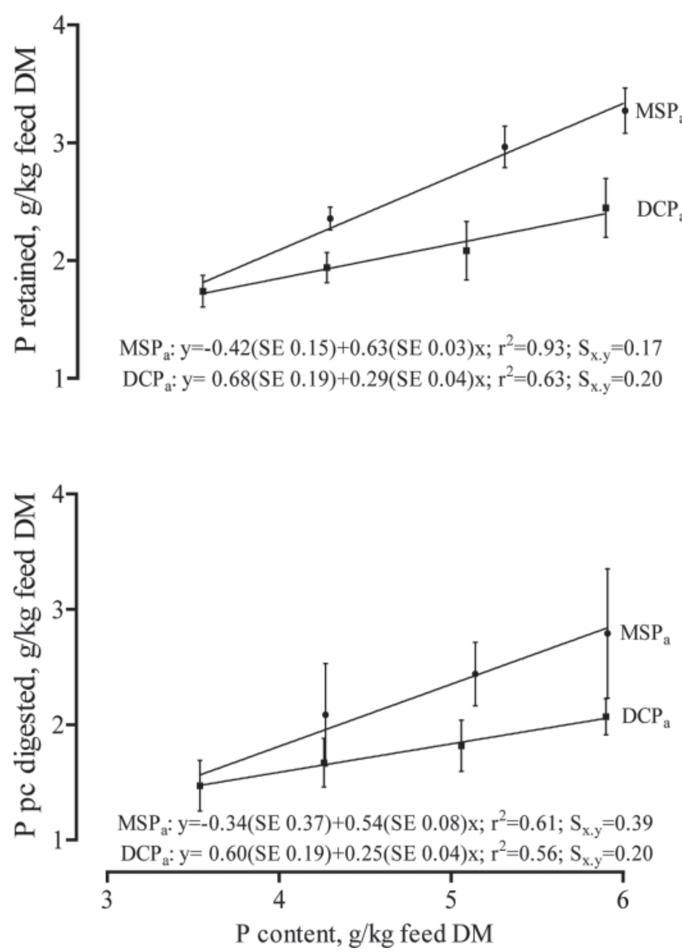

Figure 2. Phosphorus retention (upper panel) and P prececal (pc) digestibility (lower panel) depending on P content of diet in period 2 (mean and SD; n = 8 replicates per treatment). MSP_a = anhydrous monosodium phosphate; DCP_a = anhydrous dibasic calcium phosphate.

Table 4. Body weight gain, feed intake, and feed per gain ratio of broilers at different ages fed the low-P basal diet or diets supplemented with 2 different sources and levels of mineral P (n = 8 pens of 10 birds per pen)[1]

		MSP_a			DCP_a				*P*-value (ANOVA)		
Item	BD	0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	Pooled SEM	P source	P level	Level × source
d 11 to 21											
BW gain, g	342	367	430	427	388	394	439	13.4	0.748	<0.001	0.738
Feed intake, g	512	511	574	568	564	579	607	16.7	0.472	0.004	0.670
Feed/BW gain, g/g	1.50	1.39	1.33	1.34	1.46	1.44	1.38	0.02	0.386	0.004	0.720
d 25 to 35											
BW gain, g	832	837	888	925	877	870	867	23.7	0.053	0.099	0.043
Feed intake, g	1,421	1,413	1,484	1,477	1,475	1,482	1,457	25.6	0.101	0.373	0.115
Feed/BW gain, g/g	1.71	1.69	1.68	1.61	1.69	1.71	1.68	0.03	0.471	0.161	0.391

[1]BD: basal diet; MSP_a: anhydrous monosodium phosphate; DCP_a: anhydrous dibasic calcium phosphate.

BD was 63.8% and 47.4% in periods 1 and 2, respectively. P source and P level had a significant effect on pc digestibility of Ca in period 1 ($P = 0.049$ and 0.002, respectively). In period 2, there was a significant interaction ($P = 0.013$) between the level and the source of P for pc digestibility of Ca. The P level and P source alone also had a significant effect ($P < 0.007$) on this value.

The relationship between intake and pc-digested P in period 1 was linear (Figure 1, lower panel), and pc digestibility for MSP_a and DCP_a, determined by linear regression analysis, was 67% and 30%, respectively. The differences between the slopes for MSP_a and DCP_a were significant ($P < 0.001$). In period 2, the response was similar to that in period 1 (Figure 2, lower panel). Retention of P from MSP_a and DCP_a was 54% and 25%, respectively. Again, the differences between the slopes for MSP_a and DCP_a were significant ($P = 0.002$).

The differences between the slopes for MSP_a or DCP_a retention and pc digestibility in period 1 (0.70 vs. 0.67 and 0.29 vs. 0.30, respectively) and period 2 (0.63 vs. 0.54 and 0.29 vs. 0.25, respectively) were not significant ($P > 0.05$).

DISCUSSION

Experimental Approach

According to Eeckhout and de Paepe (1997), regression analysis is the most convenient approach for comparisons, because the ratio of the calculated slopes allows for a direct comparison of the phosphates involved. It is also possible to compare different criteria of P evaluation using the slopes of linear regressions. The slopes for MSP_a (0.67) and DCP_a (0.30) pc digestibility in period 1 had a ratio of 2.23, which was close to the slope ratio (2.41) determined for P retention (0.70 and 0.29, respectively). In period 1, the differences between the slopes for retention and pc digestibility for either MSP_a or DCP_a (0.70 vs. 0.67 and 0.29 vs. 0.30, respectively) were not significant. Hence, a common slope for both data sets within each P source could be calculated, and the determined pooled slopes in period 1 were 0.68 (MSP_a) and 0.29 (DCP_a).

Differences between pc digestibility and retention of P could occur as a consequence of either P excretion with urine or postileal absorption and secretion of P. Manangi and Coon (2006) used 40- and 50-d-old colostomized broilers to study the effect of different dietary nPP levels on urinary P excretion. They found that urinary excretion of P remained constant and very low from 0.08% to 0.28% dietary nPP in 40-d-old birds (6.0 ± 3.2 mg/d) and 0.08% to 0.21% dietary nPP in 50-d-old birds (1.9 ± 3.5 mg/d). Supplements above these thresholds caused an increase in urinary P excretion and indicated P supply above requirement. In our study, the maximum increase in dietary nPP was 0.33%, which was higher than the threshold levels determined by Manangi and Coon (2006). However, broilers in our studies were younger and thus required a

Table 5. Prececal (pc) digestibility of P and Ca at different ages of broilers fed the low-P basal diet or diets supplemented with 2 different sources and levels of mineral P (n = 8 pens of 10 birds per pen)[1]

		MSP_a			DCP_a				*P*-value (ANOVA)		
Period	BD	0.08%	0.16%	0.24%	0.08%	0.16%	0.24%	Pooled SEM	P source	P level	Level × source
Period 1											
pc P digestibility, %	54.1	55.1	60.8	58.2	45.7	46.3	43.7	1.4	0.730	0.705	0.093
pc Ca digestibility, %	63.8	62.9	59.1	55.6	53.7	50.6	50.6	1.6	0.049	0.002	0.199
Period 2											
pc P digestibility, %	41.5	48.8	47.5	47.2	39.2	35.9	35.1	2.4	0.839	0.361	0.694
pc Ca digestibility, %	47.4	58.8	58.7	35.0	45.9	37.5	35.1	2.7	0.007	<0.001	0.013

[1]BD: basal diet; MSP_a: anhydrous monosodium phosphate; DCP_a: anhydrous dibasic calcium phosphate.

higher P concentration in the diet. Further, they were fed at a restricted rate in the retention trials. If the urine had been relevant for P excretion, then P retention should have been lower than pc digestibility, which was not the case. Therefore, we assume that the threshold for urinary P excretion was not exceeded and urinary P losses remained negligibly low. In nonruminants, absorption of Ca and P occurs primarily in the small intestine (Veum, 2010). Thus, although not measured, there is good evidence to assume that in our study, no or only very little P absorption occurred posterior to the ileum. The study of Biehl and Baker (1997) can be taken as further evidence for the absence of relevant postileal P absorption. They found no differences in tibia ash between cecectomized and intact chicks. This suggests that inorganic phosphate, although released from inositol phosphates by microbial activity in the ceca, was not absorbed (Kerr et al., 2000).

The results in period 2 were similar to period 1. The slope ratio for MSP_a and DCP_a pc digestibility (2.16) again was very close to the P retention slope ratio of 2.17. However, the percentage values of MSP_a and DCP_a retention were on a somewhat higher level than for pc P digestibility. This may have been due to different reasons. First, feed intake in the digestibility trial was 40% higher than in the retention trial, whereas this difference was less than 20% in period 1. Consumption of P from both the BD and supplemented P sources therefore was higher, which might have brought P supply close to the level of requirement in the digestibility but not in the retention trial. Second, dietary P restriction stimulates synthesis of 1,25-dihydroxycholecalciferol in the kidney (Tenenhouse and Martel, 1993), which increased Na^+-dependent phosphate transport in the brush border membrane of the chick enterocyte (Matsumoto et al., 1980). Therefore, the active P_i absorption in the small intestine might have been increased in the retention trial compared with the digestibility trial due to a more pronounced P restriction. And third, the higher intake and digesta passage may have affected the site of absorption in the small intestine, as suggested by Kluth et al. (2005) for amino acid digestibility studies. However, all 3 hypotheses need further clarification because they were not studied in the present trials.

Age Effects

There was no significant difference between 2 age periods in the slopes for MSP_a (70 vs. 63%, $P = 0.238$) or DCP_a retention (29 vs. 29%, $P = 0.952$) and for MSP_a (67 vs. 54%, $P = 0.130$) or DCP_a pc digestibility (30 vs. 25%, $P = 0.322$). Similarly, Rodehutscord et al. (2003) did not detect differences in P utilization between 3- and 5-wk-old Pekin ducks. However, P retention and pc digestibility of the BD in the present study declined from 54% (period 1) to 49% (period 2) and from 54% (period 1) to 42% (period 2), respectively. This is in agreement with Yan et al. (2005), who reported a decrease in pc digestibility of P in broilers fed a low-P diet (0.6% Ca and 0.30% nPP) from 57% at 23 d to 47% at 32 d of age. Fonolla et al. (1981) also found a significant decline in total P retention (0.53–0.61% dietary P on DM basis) with increasing age of broilers. Percentage retention of Ca and P can be expected to be higher in younger birds because of bone formation processes (Fonolla et al., 1981). Moreover, feed consumption and P intake considerably increase with aging of birds. Therefore, the lower P retention values in the older birds may be attributed to a higher feed and P intake, and in comparison to the younger birds, a slower skeletal growth (Williams et al., 2000). The lower P retention and pc digestibility in older birds also might reflect a decline in phytate breakdown. Yan et al. (2005) observed a decrease in the release of phytate P from 40% at d 23 to 16% at d 32 of age in broilers fed a low P diet (0.6% Ca and 0.30% nPP).

Mineral P Sources

Our retention and pc digestibility values obtained by linear regression analysis were lower than those reported in the literature. Van der Klis and Versteegh (1996) reported retention values of 92% for MSP × H_2O and 55% for DCP_a in 3-wk-old broilers. Ketels and De Groote (1988) reported pc digestibility of P from DCP_a and DCP × H_2O at 3 wk of age to be 67% and 73%, respectively. The discrepancies between our study and these 2 studies may be attributed to differences in the hydration state (for MSP), experimental conditions, or methods. The hydrous MSP has a higher P availability than its anhydrous form (De Groote and Lippens, 2002). Hydrated DCP also had higher availability than its anhydrous form in broilers (Ketels and De Groote, 1988; Van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997). Van der Klis and Versteegh (1996) used a purified BD (0.2 g of P/kg) for their investigations. The experimental diets were standardized at 1.8 g of available P/kg and 5 g of Ca/kg. Ketels and De Groote (1988) used a BD that also was very low in available P. In the present study, we used a corn-soybean meal-based diet (0.35% P of feed DM), which was supplemented with 3 graded P levels. In young turkeys, Grimbergen et al. (1985) determined pc digestibility of P from DCP_a to be 35%, which confirms the values found in the present study with broilers. Leske and Coon (2002) demonstrated that the retention of P from different P sources depends on the amount of the P source included in a corn-soybean meal-based diet. In their study, P retention from a reagent-grade monocalcium phosphate declined from 98% to 59% when nPP was increased from 0.16% to 0.45%. The same tendency was observed in the present study. For instance, in period 1, MSP_a retention values were 81%, 78%, and 70% calculated by separate regression for only one (0.08%), 2 (0.08% and 0.16%), or 3 (0.08%, 0.16%, and 0.24%) inclusion levels, respectively. Wasserman and Taylor (1973) showed that with increasing concentration of P, the rate of ^{32}P absorption from specific ligated intestinal segment tended to decrease. The authors suggested the existence of a saturable component in P absorption.

Using linear regression analysis for assessing the availability of a supplement is generally assumed to avoid possible interactions between the BD and a supplement. However, this may be different in P supplementation studies where supplemented mineral P can influence phytate hydrolysis from the BD, as shown in broilers (Van der Klis and Versteegh, 1996) and in rats (Moore and Veum, 1982; Miyazawa and Yoshida, 1991), and thus the calculated P retention or pc digestibility values of the mineral phosphate source. The usage of synthetic phytate-free diets in the evaluation of P availability allows eliminating such possible interaction, but this implies the risk that data are not relevant to the poultry industry where phytate-containing diets are widely used. Kornegay (2001) estimated that the retention of P from several feed-grade P sources was on average 46% for broilers and turkeys, which is much lower than the values obtained with purified diets by suboptimal P supply under standardized conditions (Van der Klis and Versteegh, 1996; De Groote and Huyghebaert, 1997).

We conclude that both retention and pc digestibility are suitable for assessing the availability of mineral phosphate sources in broilers. The ranking of phosphates is the same based on both approaches. Comparison of results with P sources studied herein and values from the literature suggest that methodological details have a great effect, and further standardization of the protocol is needed to achieve better comparability of results. Broilers at the age of 3 and 5 wk do not greatly differ in their mineral P availability values, but further studies on this aspect also including broilers that are younger than 3 wk are suggested.

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