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Effects of phytase supplementation on phosphorus retention in broilers and layers: A meta-analysis

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ABSTRACT Phytase, a widely used feed additive in poultry diets, increases P availability and subsequently reduces inorganic-P supplementation and P-excretion. Phytase supplementation effect on P-retention in poultry has been investigated, but the effect sizes were highly variable. The present study's objective was to conduct several meta-analyses to quantitatively summarize the phytase effect on P-retention in broilers and layers. Data from 103 and 26 controlled experiments testing the phytase effect on P-retention were included in 2 separate meta-analyses for broilers and layers, respectively. The mean difference calculated by subtracting the means of P-retention for the control group from the phytase-supplemented group was chosen as an effect size estimate. Between-study variability (heterogeneity) of mean difference was estimated using random-effect models and had a significant effect ($P < 0.01$) in both broilers and layers. Therefore, random-effect models were extended to mixed-effect models to explain heterogeneity and obtain final phytase effect size estimates. Available dietary and bird variables were included as fixed effects in the mixed-effect models. The final broiler mixed-effect model included phytase dose and Ca-to-total-P ratio (Ca:tP), explaining 15.6% of the heterogeneity. Other variables such as breed might further explain between-study variance. Broilers consuming control diets were associated with 48.4% P-retention. Exogenous phytase supplementation at 1,039 FTU/kg of diet increased P-retention by 8.6 percentage units on average. A unit increase of phytase dose and Ca:tP from their means further increased P-retention. For layers, the final mixed-effect models included dietary Ca, age, and experimental period length. The variables explained 65.9% of the heterogeneity. Layers receiving exogenous phytase at 371 FTU/kg were associated with a 5.02 percentage unit increase in P-retention. A unit increase in dietary Ca from its mean increased P-retention, whereas an increase in the experiment length and layer's age decreased P-retention. Phytase supplementation had a significant positive effect on P-retention in both broilers and layers, but effect sizes across studies were significantly heterogeneous due to differences in Ca contents, experiment length, bird age, and phytase dose.

Key words: broiler, layer, phosphorus retention, phytase

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INTRODUCTION

Fifty to 85% of P stored in cereal grain is bound in phytic acid and its salts (Ravindran et al., 1995; Tran and Skiba, 2005). Therefore, a considerable amount of P in poultry diets is in the form of phytate P (**PP**), with negligible availability (Selle and Ravindran, 2007). This is because poultry have limited ability to use PP. Phytase, a digestive enzyme catalyzing the release of P from the phytate complex, is not in sufficient amount (Applegate et al., 2003). The low release of PP in the gut leads to, first, the need for greater dietary supplementation with inorganic P to meet the bird's requirement for P, and second, elevated levels of PP being excreted in manure. Phosphorus is an important mineral because it plays a major role in many body functions and in mineral deposition in the skeleton together with Ca. Also, P in poultry manure can cause environmental problems such as surface water eutrophication. Phytate can also bind to other nutrients and digestive enzymes, leading to lower nutrient digestibility and increased nutrient excretion in manure (Lenis and Jongbloed, 1999).

Given the low bioavailability of P in feed ingredients for poultry, P requirement must be satisfied by adding inorganic phosphate. However, inorganic P is an expensive mineral and a finite resource. Supplementation with commercially available exogenous phytase

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enzymes has significantly increased utilization of PP in poultry (Baxter et al., 2003; Angel et al., 2005b). However, there is wide variation in the effect of added phytase on P availability at the same phytase dose and type of diet, contributing to unreliable diet formulation (Kebreab et al., 2012). Knowledge of the size of the effect of exogenous phytase supplementation on P utilization is useful in determining the amount of inorganic P that needs to be added to meet requirement. Mathematical models help estimate such effects effectively given the size of phytase effect on P availability is accurately quantified (Kebreab et al., 2007). The effect of phytase supplementation on P retention has been investigated more frequently than that on P digestion because obtaining measures of true nutrient digestibility is challenging. Phosphorus retention measures, however, can be directly related to P excretion; therefore, determining the size of phytase effect on P retention can lead to accurate prediction of P excretion.

Meta-analyses compare and combine treatment (e.g., phytase supplementation) effect sizes obtained from numerous experiments conducted under different conditions. Moreover, meta-analyses using random-effect models allow for estimating heterogeneity of effect sizes and provide an opportunity to explore factors explaining this heterogeneity. Knowledge about the relationship between such factors and phytase effect on P retention could further improve models aiming to predict P excretion from poultry. Due to growing environmental pressures, policies and regulations that limit the use of manure containing high amounts of P are being implemented worldwide. For example, the Canadian government has taken a comprehensive approach that aims to reduce the negative impact of nutrient loading arising from manure application (Action Plan for Clean Nutrient Management Act, 2002, Ontario, OMAFRA, 2002). Poultry manure is a concern as it contains more P (1.4% of fresh weight) compared with cattle manure (0.1–0.7% of fresh weight; Mullins, 2009).

The objectives of this study were to 1) summarize the size of the exogenous phytase supplementation effect on P retention in broilers and layers and 2) estimate and explore heterogeneity of the effect sizes in both groups using meta-analytical approaches.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not required for this study because all data were obtained from the literature.

Data Sources

A search was conducted for articles published until 2013 using Science Direct, CAB direct (CAB International, Wallingford, UK) and the *Poultry Science* (http://ps.oxfordjournals.org/) online database with search terms: "phytase," "phosphorus retention," and "broilers" or "layers." The searches collectively resulted in 286 and 29 research articles for broilers and layers, respectively (after duplicates were excluded). To be included in the meta-analyses, the studies were required to have the following characteristics: 1) be in vivo poultry studies including a control treatment group that did not receive exogenous phytase; 2) be published in English, 3) report mean P retention: $(P_{intake} - P_{(excreta)})/(P_{intake}) \times 100\%$, or apparent total tract P availability in both the control and treatment groups with measures of sample variance (SD or SE) along with sample size (n). The equation for P retention has been used for both broilers and layers, meaning that the P retention includes the P retained in egg for layers; and 4) provide information on diet ingredients and nutrient composition, phytase dose, age of birds, study duration, and feed intake. Consequently, all review articles (n = 23), articles published in other languages (n = 5), and articles on studies of other species (n = 74) were excluded from the preliminary collation for broilers. Another 46 articles were excluded because they related to noncontrolled studies or did not provide the SD or SE of P retention measures, basal diet ingredients or nutrient composition (or both), and phytase dose. An additional 85 studies were excluded because they did not include P retention measures. In the end, 53 studies were used in the meta-analysis on broilers.

From the 29 studies on layers, 11 were removed because P retention was not reported. Two studies were not published in English and 4 studies lacked information regarding uncertainty of the P retention measures. Therefore, the final data set for layers consisted of 12 studies. Some studies (e.g., Keshavarz, 2000a) involved particular treatments (e.g., graded levels of dietary Ca or CP) with and without phytase supplementation. Each such test was considered a separate controlled experiment. For example, the study by Manangi and Coon (2008) investigated the effects of 2 levels of dietary Ca and 8 levels of nonphytic P on P retention using a 2 × 8 factorial treatment design. Because each of the 16 treatment combinations was tested with and without phytase, these were included as 16 separate experiments in the present meta-analysis (Figure 1). Finally, 103 experiments from 53 articles dating from 1997 to 2013 and 26 experiments from 12 articles dating from 1998 to 2011 were used in the meta-analyses for broilers and layers, respectively.

The database constructed contained mean P retention and corresponding SD for the control and phytase-supplementation treatment groups. Other variables included in the final data sets were nutrient content (DM basis) and ingredient composition (% total diet weight) of the experimental diets, including corn, soybean meal (**SBM**), DL-Met, CP, Ca, total P (**tP**), nonphytase P (**NPP**) content, and feed intake (**FI**, g/d per bird). Moreover, phytase dose (**FTU**/kg of diet), age of birds at the beginning of the experiment (in days for broilers and weeks for layers), length of the experimental period (d), and number of experimental units (N) were also incorporated. The main phytase types used in broiler and

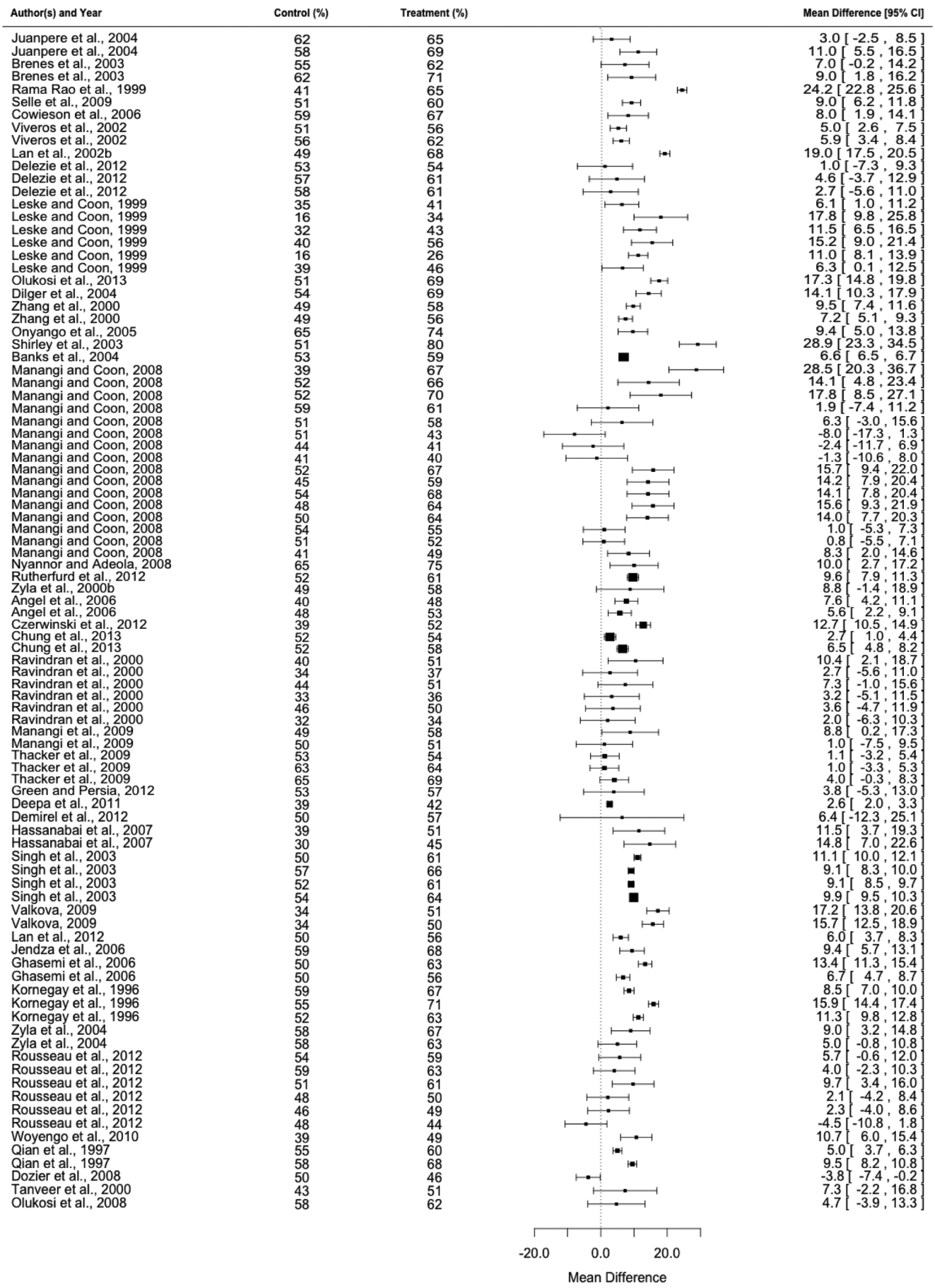

Figure 1. Forest plot showing mean P retention (% of P intake) in control and supplemented diet (treatment) groups along with mean difference (MD, boxes) and its 95% of CI (whiskers) for broilers. The dotted line represents a 0-standardized mean difference.

layer diets were 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26).

The ratio of Ca to tP (**Ca:tP**) was calculated according to dietary Ca and tP contents. In the majority of studies, SD was reported as a pooled-measure across treatment and control groups. So, similar uncertainty of P retention measures was assumed for both control and phytase treatment groups.

Statistical Analysis

Effect Size. Two separate meta-analyses were carried out to summarize the effect size of phytase supplementation on P retention in broilers and layers using the metafor package (version 1.9–1) in R (version 0.97.551, R Foundation for Statistical Computing, Vienna, Austria). Mean difference (**MD**) was chosen as the effect size estimate, which was calculated by subtracting mean P retention for the control (**CTL**) group from mean P retention for the phytase-supplemented group (**PHY**):

$$\text{MD} = \text{PHY} - \text{CTL}.$$

Mean difference allows easy effect size interpretation because it is in the original units of the response variable in question. Under the assumption of perfect control conditions, a positive MD indicates an increased P retention for phytase supplementation.

Models. Heterogeneity associated with the phytase supplementation effect on P retention was first quantified using random-effect models. In developing the random-effect models, we assumed that

$$y_i = \theta_i + e_i,$$

where y_i = the observed effect size or MD for the ith study; θ_i = corresponding true effect size for the ith study, which is unknown; and e_i = the sampling error [$e_i \sim$ N (0, sampling variance)], assumed to be known and taken as the squared SE of the effect size. It leads to the random-effect models given by

$$\theta_i = \mu + \mu_i,$$

where θ_i = true effect size (MD) in the ith study; μ = overall true effect size, and μ_i = random deviation from the overall effect size [$\mu_i \sim$N (0, τ^2)], which was estimated from data. The term τ^2 indicates heterogeneity. The random-effect models were extended to mixed-effect models including fixed effect of factors that may explain heterogeneity. The mixed-effect models are given by

$$\theta_i = \beta_0 + \beta_1 x_{i1} + \ldots + \beta_p x_{ip} + \mu_i,$$

where β_0 = overall true effect size; x_{ij} = the value of the jth explanatory variable (j = 1, 2, …, p) for the ith study; and β_j = change in the true effect per unit increase in the jth explanatory variable and again $\mu_i \sim$N (0, τ^2). Here τ^2 indicates the amount of heterogeneity not explained by the variables (Viechtbauer, 2010). Corn, SBM, Met, CP, Ca, tP contents, Ca:tP ratio, phytase dose, FI, age at the start of the experiment, and length of the experimental period were used as explanatory variables that were centered on their means (Table 1) and then regressed individually against MD. The centered variable allows interpretation of the regression effects in terms of changes in a phytase effect size for a unit change in an explanatory variable from its mean.

Publication Bias and Influence Diagnosis. The validity and robustness of meta-analysis conclusions can be affected by publication bias and the presence of influential cases (Sutton et al., 2000; Viechtbauer and Cheung, 2010). Influential cases were recognized using DFLBETA values, Cook's distance values, and the estimates of τ^2 obtained when each study is removed from the data set as described previously (Viechtbauer, 2010). Initially, 53 studies were chosen for the broiler

Table 1. Summary statistics for the explanatory variables

	Broilers				Layers			
Variable[1]	Mean	CV[2]	Minimum	Maximum	Mean	CV[2]	Minimum	Maximum
Corn (% of diet)	39.2	0.62	0.0	72.1	56.1	0.32	0.0	67.7
Soybean meal (% of diet)	26.7	0.39	0.0	41.5	19.4	0.40	0.0	38.0
DL-Met (% of diet)	0.20	0.41	0.0	0.44	0.21	0.95	0.07	0.80
ME (MJ/kg of diet)	10.4	0.30	5.0	19.1	10.5	0.24	4.8	12.4
CP (% of DM)	21.5	0.07	18.2	26.3	16.7	0.09	13.0	18.8
Calcium (% of DM)	0.80	0.29	0.39	1.39	3.66	0.09	3.10	4.20
Total P (% of DM)	0.51	0.27	0.18	0.95	0.53	0.33	0.18	1.13
NPP (% of DM)	0.24	0.41	0.02	0.50	0.19	0.43	0.05	0.40
Ca:tP ratio	1.6	0.3	0.8	3.5	8.0	0.5	3.6	22.2
Phytase dose (FTU/kg of diet)	1,039	1.25	250	12,000	371	0.31	300	600
Feed intake (g/d per bird)	78.8	0.43	21.6	207.0	98.2	0.40	46.3	115.0
Age at the start in days (broiler) and weeks (layer)	14	0.91	1	42	35	0.39	20	56
Duration of feeding period (d)	17	0.68	3	49	92	0.62	7	224

[1]NPP = nonphytase P; tP = total P; FTU = phytase units.

[2]CV = SD/mean.

meta-analysis, but the influence analysis removed some studies from the data set, leaving 45 broiler studies. Thus, 93 experiments from 45 articles and 25 experiments from 12 articles were used in the meta-analyses for broilers and layers, respectively. Publication biases of the phytase effect on P retention in broilers and layers were assessed using Egger's regression test for funnel plot asymmetry (Viechtbauer, 2010).

Model Fitting and Selection. The random-effect models were initially fitted using the restricted maximum likelihood estimation method to estimate heterogeneity (τ^2). Statistical significances of τ^2 were obtained using chi-squared tests (Higgins and Thompson, 2002). Moreover, I^2 statistics were calculated expressing τ^2 as a percentage of total variance (τ^2 + sample variance) and tested. Hence, the I^2 statistic represents the proportion of total variation in the estimate of treatment effect that is due to heterogeneity. The mixed-effect models were then constructed by including individual explanatory variables. Full mixed-effect models carrying all explanatory variables having effects ($P < 0.10$) when fitted individually were then fitted using the maximum likelihood method. Multi-collinearity was considered when selecting variables for the models. For example, tP and NPP were not analyzed together because they were highly correlated (r = 0.71). Reduced models were formed via stepwise elimination of one variable at a time and fitted again using the maximum likelihood method. The final mixed-effect models were chosen by testing reduced models versus full models using log-likelihood ratio tests. The parameter estimates of the final model were obtained by fitting the model using the restricted maximum likelihood method. Distinct sets of multivariate mixed-effect models were tested for the phytase effect on P retention for broilers and layers separately.

RESULTS AND DISCUSSION

Mean P retention in the control and phytase supplementation treatment groups and corresponding MD estimates for broilers and layers are presented as forest plots in Figures 1 and 2, respectively. Phytase supplementation consistently had a positive effect on P retention in both broilers and layers (Figures 1 and 2), but the effect sizes were variable across and within studies. Broilers were supplemented with a relatively greater dose of phytase compared with layers (mean 1,039 vs. 371 FTU/kg of diet respectively, Table 1). Moreover, broiler diets contained less corn compared with layer diets (39 vs. 56% diet), but had twice as much SBM relative to the amount of corn. The percentage of SBM when both ingredients are summed was 40 and 26% for the broilers and layers, respectively. Consequently, broiler diets were composed of more CP than layer diets (Table 1). On the other hand, layer diets contained more Ca than broiler ones, whereas P levels remain similar across both. Hence, layer diets were characterized by greater Ca:tP ratios. There is a close relationship between P and Ca in layers producing eggs. Calcium is the major structural element in eggshell and large amounts of Ca are required to synthesize the shell (Kebreab et al., 2009). Calcium and P are stored together in bone and accessed by layers during periods of low Ca status. The balance between P and Ca for mineral deposition in bone must be maintained for effective P retention. In line with the length of total production cycles, phytase was supplemented for a longer duration in layers than in broilers (92 vs. 17 d, respectively).

Random-Effect Models and Phytase Supplementation

Random-effect model analyses revealed that the effect of phytase supplementation on P retention was associated with significant ($P < 0.0001$) heterogeneity in both broilers and layers. The I^2 statistics showed that more than 95% of the total variance of the phytase supplementation effect was due to heterogeneity (Table 2). Estimates of total amount of heterogeneity (τ^2) were similar for broilers and layers (30.8 vs. 37.0). Funnel plots constructed using random-effect models were used to assess publication bias. Funnel plots in Figure 3 show the MD estimates of P retention versus the corresponding SE measures. A vertical line is drawn at zero with a CI region given by ±1.96 SE (Viechtbauer, 2010). It assumes that studies with larger sample sizes will be found near the average, and studies with smaller sample sizes will be spread on both sides of the mean. Thus, in the absence of publication bias, the majority of the points would be expected to fall inside the confidence region of the funnel plot.

Besides visual assessment, the Egger's regression test was used to assess funnel plot asymmetry. Asymmetrical funnel plots indicate the presence of publication bias. The Egger's regression test showed the funnel plots to be significantly asymmetrical ($P < 0.01$; data not shown), suggesting the presence of substantial publication bias in the random-effect models for both broilers and layers. However, publication bias is one of several factors that influence funnel plot shape. Heterogeneity also alters funnel plot shape significantly (Rothstein et al., 2005). Given the significant heterogeneity estimates mentioned above, mixed-effect models were constructed to explain heterogeneity.

Explanatory Variables and Phytase Effect Size

The efficacy of microbial phytase is influenced by several factors including dietary Ca content, bioavailability of P in the diet, age of the animal, vitamin D content, and phytase dose (Kornegay et al., 1996; Kornegay, 2001; Sebastian et al., 1996; Qian et al., 1997). Hence, related dietary and other variables available in the database were chosen as potential explanatory variables to be included in the mixed-effect models. The

Table 2. Number of studies used for the analysis (n), control group mean, heterogeneity (τ^2), τ^2 as a percentage of total variability (I^2), and total variance from random-effect models[1]

	Random-effect model				
Item	n	Mean ± SE[2]	τ^2	I^2 (%)	Total variance[3]
Broilers	93	48.4 ± 0.3	30.8	98.1	31.4
Layers	25	32.0 ± 6.8	37.0	95.6	38.7
	Mixed-effect model				
	Effect size		Heterogeneity		
	Mean ± SE	*P*-value	τ^2	*P*-value	Total variance
Broilers					
Intercept	8.60 ± 0.60	<0.001	26.0	<0.001	26.6
Phytase dose, 100 FTU/kg	0.12 ± 0.05	0.008			
Ca:tP ratio	3.02 ± 1.34	0.024			
Layers					
Intercept	5.02 ± 1.08	<0.001	12.6	0.002	14.2
Ca (% of DM)	8.15 ± 3.19	0.011			
Starting age, 10 d	−2.93 ± 0.87	0.001			
Duration, 10 d	−0.47 ± 0.15	0.002			

[1]Mixed-effect models also include estimates of overall P retention (% of P intake, intercept), effect of explanatory variables, heterogeneity and total variance estimates. tP = total P; FTU = phytase units.

[2]Mean P retention (% of P intake) for the control diets.

[3]Total variance = total heterogeneity estimates (τ^2) + within-study variability.

balance between Ca and P are important because they have an antagonistic relationship. Increasing dietary Ca reduces P absorption and also reduces utilization of PP (Edwards and Veltman, 1983; Davis, 1959; Waldroup et al., 1963; MacDonald and Solvyns, 1964; Kondos and McClymont, 1967). Hence, Ca:tP ratio was calculated and added as an explanatory variable.

Effect of Phytase on P Retention in Broilers. The explanatory variables having effects ($P < 0.10$) when fitted individually and subsequently selected for the full mixed-effect model for broilers included dietary tP, Ca:tP ratio, NPP, CP, phytase, and FI. After stepwise elimination of variables, the final mixed-effect model for broilers included phytase dose and Ca:tP ratio, indicating significant independent effects on P retention (Table 2). These variables explained 15.6% (τ^2 = 26.0 vs. 30.8) of heterogeneity and 15.3% of total variance (26.6 vs. 31.4) of the phytase effect on P retention in broilers. As the intercept estimate (Table 2) indicates, adding a mean phytase dose of 1,039 FTU/kg of diet containing a mean Ca:tP ratio of 1.6 (Table 1) increased P retention in broilers by 8.6 percentage units.

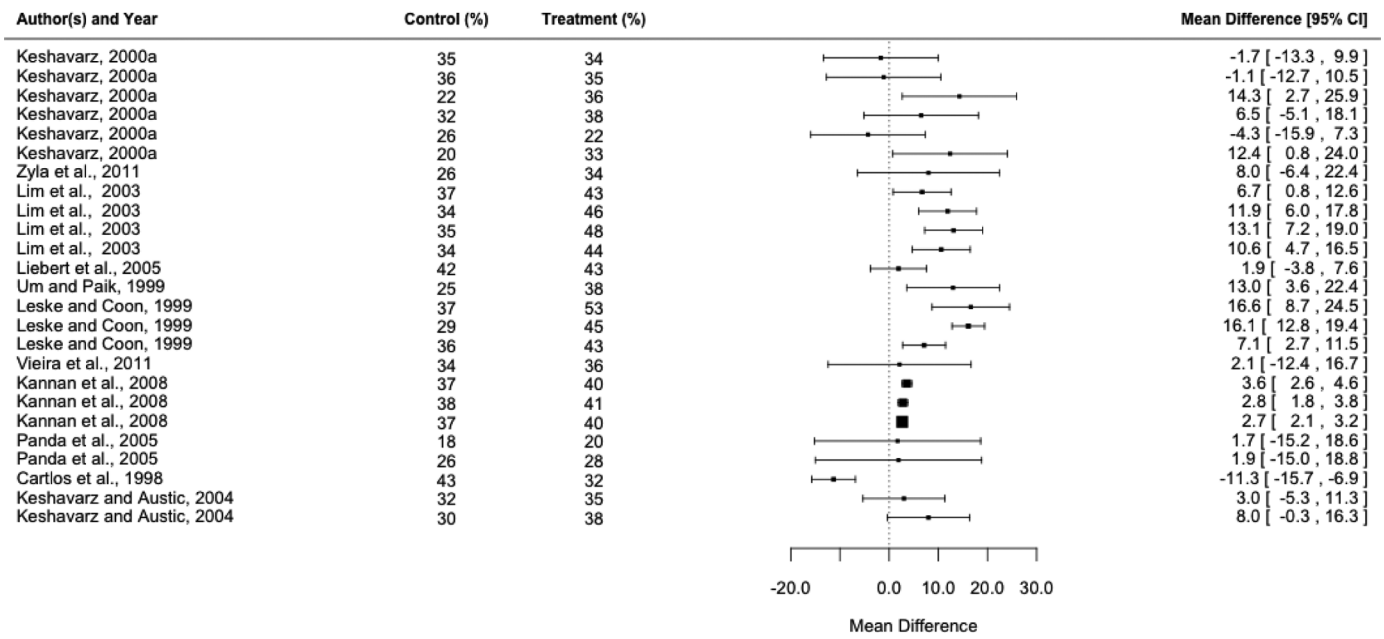

Figure 2. Forest plot showing mean P retention (% of P intake) in control and supplemented diet (treatment) groups along with mean difference (MD, boxes) and its 95% of CI (whiskers) for layers. The dotted line represents a 0-standardized mean difference.

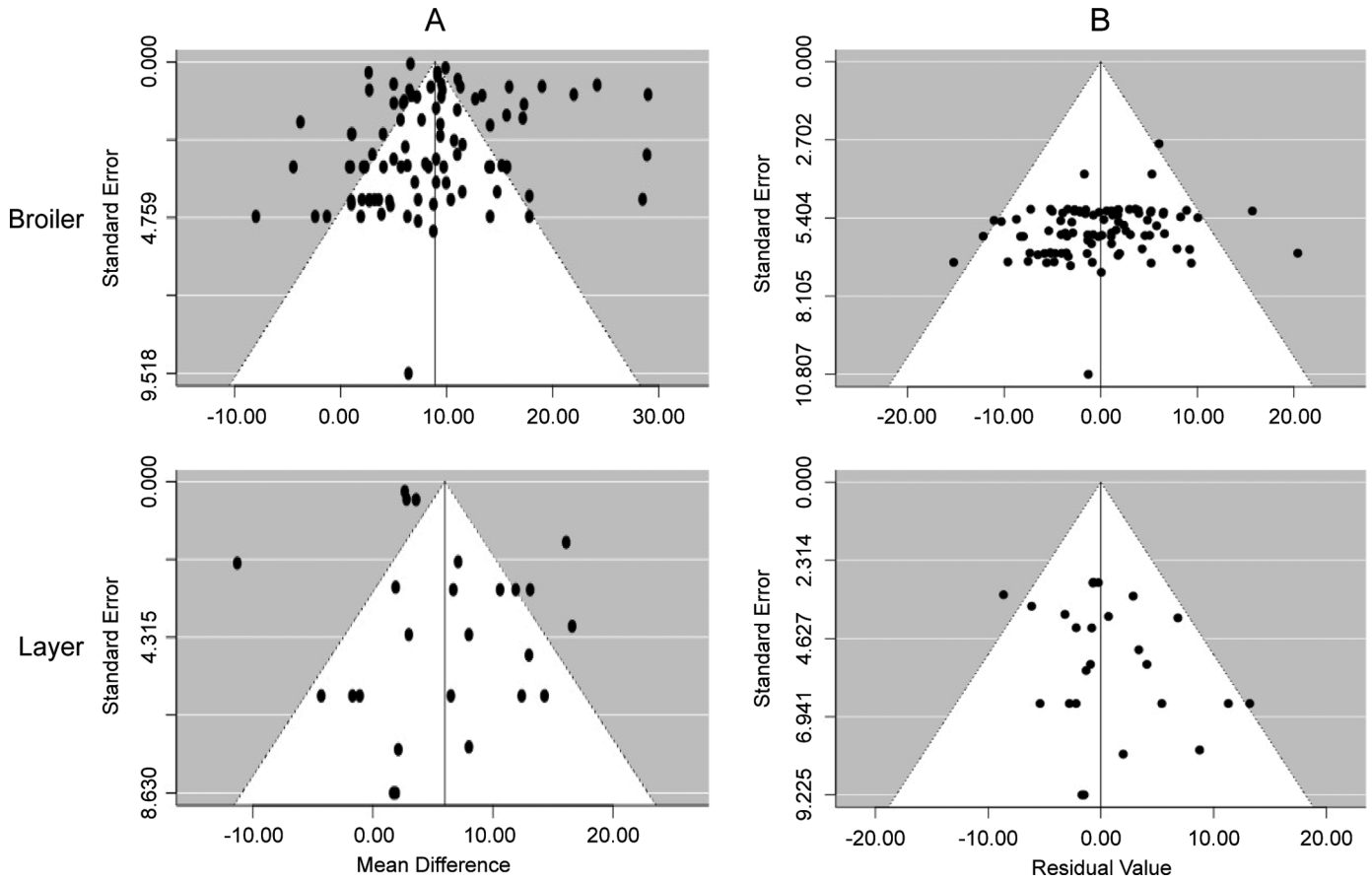

Figure 3. Funnel plots for phytase supplementation effect on P retention (% of P intake) in broilers and layers from random-effect models (A) and mixed-effect models (B).

Mean P retention in broilers receiving the same diet but not supplemented with phytase was 48.4% (Table 2). From an excretion standpoint, broilers consuming 1 g of total P excreted 520 mg into the environment when phytase was not supplemented. Supplementation of the diet with phytase reduced P excretions by 90 mg as more of the ingested P was digested and retained in the body. For each additional 100 FTU/kg increase in phytase dose and a unit increase in Ca:tP ratio, P retention increased by 0.12 and 3.02 percentage units, respectively.

The Ca:tP effect can be also interpreted as the effect of increasing Ca content or decreasing total P content in the diet. Calcium and P are closely related because Ca is stored almost entirely as hydroxyapatite crystals of Ca phosphate in bone. An increase in Ca:tP ratio has a positive effect on phytase efficacy on P retention. This may be because an increase in Ca level allows greater bone mineralization and consequently P retention. However, the nonsignificant effect ($P = 0.90$, data not shown) of dietary Ca content on phytase effect size indicates a potential negative effect of total P content, as shown in Figure 4. Also, the phytase effect numerically decreased ($P = 0.16$, data not shown) as dietary NPP content increased (Figure 4), showing that the positive effect of Ca:tP on phytase-induced P retention may be partly due to increasing the dietary NPP level. High NPP levels are masking the phytase effect on P retention, and thus, the smaller the NPP level, the higher the phytase induced P retention. It has been shown that low nonphytic acid levels lead to better efficacy of phytase with regard to P digestibility (Ravindran et al., 2000). It has also been shown that, when combined with phytase, reduced NPP level in the diet decreases litter total P (Applegate et al., 2003) and the range of phytase responses are higher. Jiang et al. (2013) also showed that phytase supplementation in low NPP diets can improve growth performance with a higher ash content in bone.

Exogenous phytase is, for the most part, active in the proximal segments of the gastrointestinal tract (crop, proventriculus, and gizzard) of poultry, where low pH levels increase the susceptibility of phytate to degradation (Campbell and Bedford, 1992). Mineral-phytate complexes, particularly Ca phytate, reduce phytase efficacy (Nelson, 1984; Angel et al., 2002) as they can directly decrease phytase activity by competing for the active sites of the enzyme (Wise, 1983; Pointillart et al., 1985). Calcium and phytate interactions occur under acidic conditions and result in soluble and insoluble complexes that could negatively affect exogenous phytase efficacy. It has been reported that dietary Ca:tP ratios increasing from 1.0 to 1.4 reduce the efficiency of supplemental phytase (Qian et al., 1997). Qian et

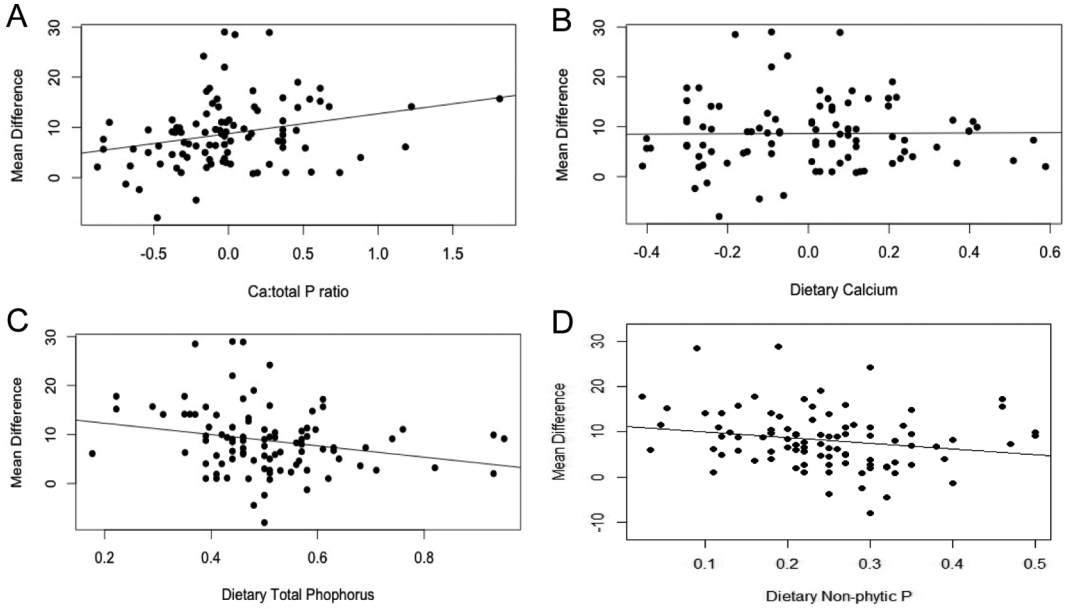

Figure 4. Mean difference between P retention in the phytase-supplemented group and control group with changing dietary Ca to total P ratio (A), dietary Ca content (% DM, B), dietary total P content (% DM, C), and dietary nonphytic P content (% DM, D) in broilers.

al. (1997) also showed that P retention is increased by phytase addition but is negatively influenced by increasing the dietary Ca:tP ratio and this effect was stronger at lower levels of available P (NPP). Moreover, the amount of dietary Ca alone has been reported to negatively affect the use of phytase in poultry as it inhibits PP hydrolysis (Tamim and Angel, 2003).

The results of this study showed a positive association between Ca:tP ratio and phytase efficacy, which is contradictory to previous observations (Qian et al., 1997; Van der Klis et al., 1997). However, their observations were related to diets with narrow ranges of Ca contents and Ca:tP ratios compared with the range across the studies included in the present analyses (0.8 to 3.5, Table 1). Moreover, the studies demonstrating the negative effect of Ca:tP ratios on phytase involved smaller phytase doses (600 to 900 FTU/kg of diet; Qian et al., 1997; Van der Klis et al., 1997). In contrast, the majority of experiments included in the present analyses were associated with larger doses with a mean of 1,039 FTU/kg of diet (Table 1). To assess a potential interaction between phytase dose and dietary Ca:tP ratio, the effect of Ca:tP ratio on phytase-induced P retention was tested separately in studies using phytase dose <800 or ≥800 FTU/kg of diet, with 800 FTU/kg of diet being the median for the data set. There was no Ca:tP ratio effect ($P = 0.26$), when phytase dose was <800 FTU/kg of diet, however, there was a positive ($P = 0.05$) effect for doses ≥800 FTU/kg of diet (data not shown).

The hydrolysis rate of PP depends on phytase enzyme concentration. Phytic P release varies from 31 to 58% when the phytase dose is raised from 250 to 1,000 FTU/kg of diet (Denbow et al., 1998). Moreover, several authors have shown that increasing phytase dose leads to a positive impact on P retention in broiler diets (Sebastian et al., 1996; Yi et al., 1996; Qian et al., 1997; Ravindran et al., 2000; Zyla et al., 2000a; Lan et al., 2002a; Viveros et al., 2002; Shirley and Edwards, 2003; Dilger et al., 2004; Onyango et al., 2004; Zyla et al., 2004; Onyango et al., 2005). However, the bird's performance reaches a plateau when the phytase level is around 500 to 1,000 FTU/kg of diet (Simons et al., 1990; Denbow et al., 1995). In contrast, Shirley and Edwards (2003) reported that birds consuming up to 12,000 FTU/kg of diet can achieve maximum performance and that P retention significantly increases as dietary phytase levels increases gradually from 0 to 12,000 FTU/kg of diet. These results are consistent with results of the present study, which show P retention increased by 0.12 percentage units for each 100 FTU/kg of diet increase in phytase dose. However, we did not observe a P retention plateau as reported by Shirley and Edwards (2003).

The selected variables in the final mixed-effect model could only explain 15.6% of heterogeneity with a significant amount ($P < 0.001$, Table 2) left unexplained. Variables such as bird characteristics including breed and health status, and other dietary characteristics such as pelleting temperature, diet particle size, and vitamin D supplementation could explain further the heterogeneity of the phytase effect on P retention. For example, Chung et al. (2013) reported that bird breed in broilers has a significant effect on P retention. Lack of information in the articles used in the present meta-analyses preclude using such variables.

Effect of Phytase on P Retention in Layers. The explanatory variables having an effect ($P < 0.10$) when fitted individually and subsequently selected for the full mixed-effect model for layers included dietary Ca

content, phytase dose, Ca:tP ratio, age at start of the trial, and the length of the experiment. After stepwise elimination of variables, the final mixed-effect model included duration of the experiment, age at the start of the trial, and dietary Ca content, indicating significant independent effects on P retention (Table 2). These variables explained 65.9% (τ^2 = 12.6 vs. 37.0) of heterogeneity and 63.3% of total variance (14.2 vs. 38.7) of the phytase effect on P retention in layers. Supplementation with exogenous phytase of a diet having a mean Ca content of 3.66% DM for 92 d on average, starting when birds were 35 wk old, increased P retention by 5.02 percentage units. The phytase-induced P retention rise increased by 8.15 percentage units for each unit increase in dietary Ca content (Table 2). In contrast, prolonging phytase supplementation duration and age of the birds had negative effects. For each extra 10 d over the 92 d mean duration, phytase-induced P retention decreased by 0.47 percentage units. For each extra 10 d, it also decreased by 2.93 percentage units among birds that started receiving phytase when they were 36 wk old compared with birds that started 1 wk earlier. This is in agreement with Scheideler and Sell (1987) who stated that regardless of phytase supplementation, the ability of laying hens to use phytase P may decline with age. Van der Klis et al. (1997) reported that Ca and P absorption in 36-wk-old hens was significantly lower than in younger hens (24 wk old), leading to less P retention. The present results reflect a negative effect of age on efficacy of phytase with regard to P retention instead of simply an effect of phytase efficacy on P retention. The potentially negative effect of age on P absorption should occur in both control and phytase supplementation groups. Hence, the effect of age on differences in P retention between control and phytase group (MD) should be related to an effect on association between phytase and P retention. Overfeeding of dietary P is quite common on commercial farms, with excesses of 20 to 100% over requirement (Applegate and Angel, 2008).

The main site of microbial phytase activity in the digestive tract of laying hens is the crop (Al-Sharafat et al., 2009), where 69 to 86% of added microbial phytase activity has been detected (Liebert et al., 1993). This is because solubility and digestibility of P is greater, whereas in the distal parts of the gut, P solubility decreases. Thus, rates of passage from the various compartments of gastrointestinal tract, particularly the fore-stomach, need to be taken into account in calculating extent of P digestion. A bird's feed consumption is governed by several factors, including BW, which is related to age. Laying hens, compared with broilers, are older and thus have a more developed gastrointestinal tract, leading to greater feed consumption. This could lead to a higher passage rate affecting the activity of phytase on P retention.

Calcium is the major dietary divalent cation for laying hens. It can progressively precipitate the phytate by forming an extremely insoluble Ca-phytate complex in the intestine, and consequently PP, as well as Ca itself, is largely unavailable for absorption (Abdallah et al., 1993; Sebastian et al., 1996; Nahm, 2007; Singh, 2008). Our results show that dietary Ca content positively affects phytase efficacy in layers. As mentioned earlier for broilers, Ca can directly interact with PP in the gut, forming complexes that reduce phytase activity. However, this effect could not be quantified in our results. Kebreab et al. (2009) developed a dynamic model of Ca and P flows in layers, showing that P excretion in feces and urine depends on Ca supply. For eggshell formation, layers need significant amounts of Ca, which must be supplied at specific times during the day to match periods of eggshell formation. Indeed, even though eggshell formation largely occurs during the night, Ca supply during the night is low because the bird hardly eats during the dark. If there is insufficient exogenous Ca from the feed available at moments of eggshell formation when Ca is required, this will stimulate Ca mobilization from the medullary bone. Calcium is stored in bone with P in an apparent 2:1 ratio, and increased Ca mobilization from bone will result in P mobilization and subsequent excretion in feces and urine of P as well, decreasing P retention (Kebreab et al., 2009). Supplying more Ca in the feed may decrease the need to mobilize bone, also decreasing P mobilized from bone, resulting in an apparently increased P retention. This mechanism may explain the positive effect of Ca observed in the present meta-analysis. Alternatively, as described for broilers, one might explain the positive effect of Ca in an indirect way. Indeed, highly digestible inorganic P (fed with Ca) has been used in 16 experiments, which may account for the positive impact of Ca on the phytase effect on P retention in layers.

In summary, supplementation of broiler and layer diets with exogenous phytase at 1,039 FTU/kg of diet and 371 FTU/kg of diet increased P retention by 8.60 and 5.02 percentage units, respectively. Certain factors significantly affected efficacy of phytase on P retention, explaining 15.6 and 65.9% of the heterogeneity in broilers and layers, respectively. The phytase inclusion dose and Ca:tP ratio positively affect phytase-induced P retention in broilers. In layers, dietary Ca has a positive influence, whereas length and age at the start of the experiment have a negative effect. The heterogeneity could have been further explained by considering other variables representing more detailed dietary and animal characteristics, particularly in broilers. The estimated phytase effect sizes should allow improvements in mathematical models predicting P excretion from poultry.

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