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Impacts of age and calcium on Phytase efficacy in broiler chickens

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ABSTRACT

A total of 648 straight-run hatchling Heritage 56M × fast feathering Cobb 500F broiler birds were used to determine the effects of Ca concentration and age on phytase efficacy. Corn and SBM based diets with 0.19% non-phytate P were prepared with three Ca (6.5, 8.0 and 9.5 g/kg) concentrations. A 6-phytase[5] was added on-top at 0, 500 or 1000 FTU/kg at each Ca concentration, resulting in a total of 9 treatments. Broiler birds were fed the diets for 2 d either from 7 to 9 (6 birds/replicate) or 19 to 21 (3 birds/replicate) d of age, and ileal content was collected from every bird at the end of each feeding period to determine apparent ileal digestibility coefficient (**AID**). Age effect was determined by comparing responses between birds fed from 7 to 9 and 19 to 21 d of age. There was no interaction between Ca and phytase on AID P regardless of age. Increasing Ca from 6.5 to 9.5 g/kg resulted in 12 (0.58 vs. 0.51) and 11% (0.64 vs. 0.57) reduction in AID P, in 9-d-old and 21-d-old birds, respectively ($P < .05$). Compared to birds fed diets without phytase, AID P was 100 and 155% greater in 9 d old birds fed 500 and 1000 FTU phytase/kg diets, respectively ($P < .05$). Similar but lesser improvement in AID P was also seen in 21 d old birds, with 63 and 76% improvement as a result of 500 and 1000 FTU/kg phytase inclusion, respectively ($P < .05$). Despite similar pattern in response to Ca and phytase, the degree of dietary impact and efficacy of phytase was affected by age of birds. In the absence of phytase, detrimental effect of Ca was more apparent in 9 d old than 21 d old bird, where greater difference in AID P was seen when Ca increased from 6.5 to 9.5 g/kg. With phytase inclusion, differences in AID P between 9 and 21 d old birds were reduced, which was more apparent with higher phytase and Ca inclusion. Net improvement of digestible P for 500 and 1000 FTU phytase/kg was 1.55 and 2.42, 1.45 and 1.72 g/kg, respectively for 9 and 21 d old birds. It is clearly shown that, even though birds at different ages responded to Ca and phytase similarly, the detrimental impact of Ca and benefit of phytase inclusion was greater in younger than older birds.

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1. Introduction

Phosphorus (**P**) is usually the third most expensive nutrient in poultry feed following protein and energy (Potchanakorn and Potter, 1987; AgriStats 2012 end of year summary, 2013). Even though 40–60% of P presented in typical corn-soybean meal (**SBM**) based poultry diets is in the form of phytate-P (**PP**) (National Research Council, 1994), the availability of PP varies and can be affected by several factors (Applegate et al., 2003). For example, Tamim et al. (2004) showed that PP disappearance was 69.2% in birds fed a corn-soybean meal based diet without added inorganic Ca or P, whereas adding 5 g/kg of inorganic Ca from limestone decreased PP disappearance to 25.4%. Similar negative impact of dietary Ca has also been demonstrated in several other studies (Tamim and Angel, 2003; Rousseau et al., 2012; Adeola and Walk, 2013). In contrast to the negative effect of Ca, it has been well demonstrated in poultry species that utilization of PP can be significantly improved by phytase (Angel et al., 2002; Selle and Ravindran, 2007; Adeola and Cowieson, 2011).

Despite the known effects of Ca and phytase on PP utilization, the interaction between the two factors is not clear, as both neutral and negative impacts of Ca on phytase efficacy have been reported (Tamim et al., 2004, Manangi and Coon, 2008, Rousseau et al., 2012; Adeola and Walk, 2013). In part, these discrepancies can be due to different ranges of Ca tested, differences in the physical characteristics and solubility of the limestones used, as well as the specific activity and/or concentration of the phytase tested.

To date, most digestibility studies especially those related to phytase are done in 3-wk-old broilers (between 18 and 24 d of age) and rarely are any studies conducted in younger birds (< 14 d of age). Given that the intestinal tract undergoes tremendous changes during the first two weeks post hatch (Obst and Diamond, 1992; Uni et al., 1998; Uni et al., 1999), accompanied by increased digestion and absorption of dietary nutrients, differences in response to diet impact in the presence and absence of phytase are likely to exist between younger and older birds. Thus, the objectives of the current study were to: 1) determine the impact of Ca on the efficacy of a new generation *Buttiauxella* 6-phytase in 9 and 21 d-old broiler birds and 2) determine the impact of age on the responses to diet Ca and phytase on apparent ileal P digestibility.

2. Materials and methods

2.1. Animals and housing

All animal care procedures were approved by the University of Maryland Animal Care and Use Committee.

On day of hatch, 648 straight run Heritage 56M × fast feathering Cobb 500F broiler birds were obtained from a local commercial hatchery and placed in floor pen rooms with artificial light and temperature control. A commercial type starter diet with adequate nutrient concentrations (National Research Council, 1994, AgriStats 2012 end of year summary, 2013) and no exogenous phytase (225 g/kg CP, 13 g/kg digestible Lys, 13.1 MJ/kg ME_n, 10 g/kg Ca, and 4.5 g/kg non-phytate P (**nPP**)) was fed to birds until d 6. In the morning of d 7, birds (average body weight was 147 ± 1, 6 birds/pen) were placed into battery pens (Modified Petersime grower batteries, Petersime Incubator Co, Gettysburg OH) preassigned to treatment (**Trt**) based on a within block (room) randomization. Birds were weighed individually to ensure similar pen weight and to minimize within pen weight variation. The wire floored battery pens (Width × Depth × Height; 99 cm × 68 cm × 37 cm) were equipped with nipple drinkers (2 per pen) and 2 external feed troughs (Length × Width × Depth; 63.5 cm × 8.9 cm × 5.67 cm). The remaining birds were kept in the floor pens and continued to be fed the starter diet until d 19. For the birds placed in battery cages, Trt diets were fed from 7 to 9 d of age. On d 9, all birds in the battery cages were euthanized by cervical dislocation and distal ileal content was collected from all birds for digestibility determination. The distal ileum was defined as the last half of the intestinal portion between Meckel's diverticulum and the ileo cecal junction. The same procedure was applied again on d 19 (average weight was 697 ± 21 g) on the remaining birds kept in floor pens and fed the same starter diet from d 0. At 19 d, 3 birds were placed in each pen. Treatment diets were offered to from 19 to 21 d of age and distal ileal content were collected in the morning of d 21. Pen assignment remained the same between the two feeding periods to avoid potential pen effect.

Photoperiod for both battery cage and floor pen rooms was 24 light **(L)**:0 dark **(D)** from hatch to 3 d, 14L:10D from 4 to 7 d, 16L:8D from 8 to 12 d, and 18L:6D from 13 to 21 d of age. Room temperature was kept at an average of 31 °C from hatch to 3 d and brooder lamps were used to provide additional heat. Temperature was lowered by 1 °C every 2–3 d such that bird comfort was maintained and temperature was 24 °C at 21 d of age. Birds were checked twice daily and weight of dead bird was recorded. Feed and water were offered for *ad libitum* consumption throughout the trial.

2.2. Experimental design and diets

A corn and SBM mash basal diet[6] (mean diameter[7] (**d_{gw}**) = 0.854 mm; geometric SD (**S_{gw}**) = 0.576 mm) was mixed and analyzed for dry matter, macro minerals, protein, ether extract, and amino acids (Table 1). Based on the analyzed Ca and P concentrations in

[6] Particle size and distribution for diets, monocalcium phosphate and limestone were determined by ASAE method S319.3, 1997.

[7] Particle size distribution of basal diet: > 2.360 mm, 2.03%; 2.000–2.360 mm, 3.02%; 1.700–2.000 mm, 6.28%; 1.400–1.700 mm, 11.00%; 1.180–1.400 mm, 10.63%; 1.000–1.180 mm, 11.99%; 0.850–1.000 mm, 11.83%; 0.710–0.850 mm, 7.24%; 0.500–0.710 mm, 19.10%; 0.300–0.500 mm, 11.73%; 0.250–0.300 mm, 1.89%; < 0.250 mm, 3.25%.

Table 1
Ingredient and chemical composition of the basal diet.

Ingredient	Basal (g/kg, as-fed basis)
Corn	578.0
Soybean meal (48% CP)	368.7
Soy oil	34.6
Salt	5.2
Monocalcium phosphate[a]	1.3
Limestone[b]	3.9
DL-Methionine, 99%	2.8
Biolys, 55%	1.6
L-Threonine, 98.5%	0.3
Choline chloride, 25%	1.7
Mineral Premix[c]	1.0
Vitamin Premix[d]	0.8
Total	**1000.0**
Formulated (analyzed) concentrations (g/kg) (mean ± SD)[e]	
ME_n, MJ/kg	13.1
Crude protein	224.0 (250.0 ± 2.1)
Lysine	13.3 (15.0 ± 0.3)
Digestible Lysine	12.0 (13.1 ± 0.2)
Methionine + Cysteine	9.8 (10.6 ± 0.3)
Calcium (Ca)	3.0 (4.0 ± 0.2)
Total phosphorus (tP)	4.5 (4.3 ± 0.1)
Phytate phosphorus (PP)	3.0 (2.6 ± 0.1)
Non-phytate phosphorus (nPP)[f]	1.5 (1.7)

[a] Analyzed Ca and P: 160.6 g/kg and 219.5 g/kg, respectively.
[b] Analyzed Ca, 366.5 g/kg.
[c] Supplied per kg of diet: zinc from zinc sulfate, 80 mg; manganese from manganese sulfate, 100 mg; iron from iron sulfate, 20 mg; copper from copper sulfate, 3 mg; iodine from calcium iodate, 3.9 mg; selenium from selenium sulfate, 0.3 mg.
[d] Supplied per kg of diet: vitamin A, 15,111 IU; vitamin D, 5333 IU; vitamin E, 53.33 IU; vitamin B_{12}, 26.66 mg; riboflavin, 17.78 mg; niacin, 71.11 mg; pantothenic acid, 24.89 mg; vitamin K_3, 3.2 mg; folic acid, 2.13 mg; biotin, 0.142 mg; thiamine, 4.44 mg; pyridoxine, 6.22 mg.
[e] Crude protein, amino acids and PP were analyzed in duplicate, Ca and tP were analyzed in triplicate.
[f] Concentration determined based on analyzed tP minus analyzed PP.

Table 2
Formulated (analyzed) calcium (**Ca**), Phophorus (**P**), non-phytate P (**nPP**) and phytase concentrations (mean ± SD) in final diets (as is basis).[a]

Ca, g/kg		P, g/kg		nPP, g/kg[b]		Phytase, FTU/kg	
Fml[c]	Ana[c]	Fml	Ana	Fml	Det[c]	Fml	Ana
6.5	6.8 ± 0.3	5.0	4.4 ± 0.1	2.0	1.9	0	< 50
6.5	6.8 ± 0.3	5.0	4.4 ± 0.1	2.0	1.9	500	568 ± 51
6.5	6.8 ± 0.3	5.0	4.4 ± 0.1	2.0	1.9	1000	1004 ± 134
8.0	7.7 ± 0.4	5.0	4.4 ± 0.2	2.0	1.9	0	< 50
8.0	7.7 ± 0.4	5.0	4.4 ± 0.2	2.0	1.9	500	546 ± 24
8.0	7.7 ± 0.4	5.0	4.4 ± 0.2	2.0	1.9	1000	1047 ± 138
9.5	9.6 ± 0.6	5.0	4.3 ± 0.2	2.0	1.8	0	< 50
9.5	9.6 ± 0.6	5.0	4.3 ± 0.2	2.0	1.8	500	450 ± 44
9.5	9.6 ± 0.6	5.0	4.3 ± 0.2	2.0	1.8	1000	1108 ± 183

[a] Diet Ca, P and phytase concentrations for each treatment were analyzed in triplicate.
[b] Concentration determined based on analyzed total P minus analyzed phytate P in the diet.
[c] Fml, formulated concentrations; Ana, analyzed concentrations; Det, determined concentrations based on the difference between analyzed P and PP.

the basal diet, pre-analyzed limestone[8] (IMI Cal Pro, IN; $\mathbf{d_{gw}} = 0.402$ mm; $\mathbf{S_{gw}} = 0.255$ mm) and monocalcium phosphate[9] (Kirby Agri, PA; $d_{gw} = 0.759$ mm; $S_{gw} = 0.258$ mm) were added to the basal diet to achieve the desired Ca and nPP concentrations in the Trt diets. The basal diet was included in all Trt diets at the same level (96.3%) to ensure that only Ca and P were different between Trts.

[8] Particle size distribution of limestone used in the basal and Trt diets: > 1.180 mm, 0.07%; 1.000–1.180 mm, 0.71%; 0.850–1.000 mm, 5.36%; 0.710–0.850 mm, 9.74%; 0.600–0.710 mm, 10.07%; 0.500–0.600 mm, 15.96%; 0.425–0.500 mm, 10.84%; 0.355–0.425 mm, 13.10%; 0.300–0.355 mm, 9.36%; 0.250–0.300 mm, 7.88%; 0.150–0.250 mm, 10.20%; 0.075–0.150 mm, 4.99%; < 0.075 mm, 1.72%.

[9] Particle size distribution of monocalcium used in the basal: > 1.180 mm, 0.82%; 1.000–1.180 mm, 8.64%; 0.850–1.000 mm, 34.83%; 0.710–0.850 mm, 24.04%; 0.600–0.710 mm, 17.22%; 0.500–0.600 mm, 9.13%; 0.300–0.500 mm, 3.81%; < 0.300 mm, 1.51%.

Titanium dioxide (TiO_2) was added at 3 g/kg as the inert marker and Celite® (World Minerals, CA) was used as a filler to achieve 100% (Table 2).

The Trt diets were formulated to contain 6.5, 8.0 or 9.5 g/kg Ca from limestone and 0.19% nPP from monocalcium phosphate. To prepare the diets with phytase, one batch was made for each Ca concentration and divided into three equal lots to minimize potential variability. For each Ca concentration, a 6-Phytase[10] (Axtra® PHY, *Buttiauxella* spp., expressed in *Trichoderma reesei*) was then added on top, at 0, 500 and 1000 FTU/kg each to one of the three lots and mixed so that the only difference among those lots was the phytase concentration.

There were 9 Trt diets comprised of a factorial arrangement of 3 Ca x 3 phytase concentrations and each treatment was replicated 8 times. All Trt diets were fed as mash over the 2 feeding periods (7–9, and 19–21 d of age).

2.3. Measurements and sample collection

At the end of each feeding period (9 and 21 d of age), all birds in a pen were sampled and sacrificed by cervical dislocation.

Ileal samples were taken immediately after cervical dislocation from all birds. The last half of the ileum with the contents were gently expressed by flushing with cold distilled water. Digesta contents were pooled by pen, freeze dried, ground to pass through a 0.25 mm screen and stored in air tight containers at 4 °C until analyzed. Birds fed from 7 to 9 and 19 to 21 will be referred as 9 (younger) and 21 d (older) old birds, respectively.

2.4. Lab analysis

All diets were ground to pass through a 0.5 mm screen before analysis. Samples were analyzed in duplicate except where specified otherwise. Dry matter of diets and ileal contents were determined by drying overnight in a 100 °C oven (Shreve et al., 2006). Diet and ileal Ca and P were determined, in triplicate, after acid digestion and analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES; AOAC, 1999). Titanium (**Ti**) concentrations in diets and ileal contents were determined by a colorimetric method adopted from Short et al. (1996) where samples were first ashed at 580 °C and then digested in 7.4 M H_2SO_4. Crude protein and ether extract in the basal diet were analyzed, according to AOAC methods 990.03 (2003) and AOAC methods 920.39 (2003), respectively. Amino acids, except Trp, (AOAC method 994.12, 2003) and phytate-P (Ellis et al., 1977) in the basal diet were also analyzed. Phytase activities in all Trt diets were determined according to the ISO 30024 (2009) procedure where one phytase unit (FTU) is the amount of enzyme that releases 1 μmol of inorganic orthophosphate from a sodium phytate substrate per minute at pH 5.5 and 37 °C.

2.5. Determination of the age effect

In the current trial, broiler birds were fed Trt diets for only two days, from 7 to 9, or 19 to 21 d of age. From several previous trials conducted in our lab (Tamim and Angel, 2003; Tamim et al., 2004; Proszkowiec-Weglarz et al., 2013), feeding for 48 h or less minimizes minimized the ability of broiler chickens to alter P digestibility when Ca or P deficient or P and Ca imbalanced diets are fed. Thus the digestibility obtained from birds at 9 and 21 d of age reflected the inherent ability of the animal, that was fed a diet that met all requirements. to digest the nutrient. Differences in nutrient digestibility between 21 and 9 d old birds denote the age effect.

2.6. Calculations

Apparent ileal digestibility coefficient (**AID**) of Ca (**AID Ca**) and P (**AID P**) was calculated based on the following formula using TiO_2 as the inert marker:

$$\text{AID} = \frac{(Min/TiO_2)_d - (Min/TiO_2)_i}{(Min/TiO_2)_d}$$

Where $(\text{Min}/\text{TiO}_2)_\text{d}$ is the ratio of minerals (Ca or P) to TiO_2 in the diet and $(\text{Min}/\text{TiO}_2)_\text{i}$ is the ratio of minerals (Ca or P) to TiO_2 in the ileal digesta.

Digestible P was calculated as follows:

$$\text{Digestible P(g/kg)} = (AID\ _P) \times tP$$

Where tP is the analyzed total P concentration in the diet.

Phytase efficacy, expressed as net improvement of digestible P (**DigP**) due to phytase inclusion was calculated as follows:

$$\text{Net improvement(g/kg)} = DigP_{phy} - DigP_{non-phy}$$

Where DigP_Phy and $\text{DigP}_\text{non-phy}$ are the DigP in birds fed the phytase and non-phytase Trt, respectively, at the same Ca concentration and age.

Age effect was determined by the difference of AID (Ca or P) between 21 and 9 d old birds fed the same Trt diet.

[10] Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK.

Table 3
Effects of Ca and phytase concentrations on apparent ileal Ca and P digestibility coefficients (**AID Ca** and **AID P**) of broilers fed from 7 to 9, and 19 to 21 d of age.[1]

Ca[2], g/kg	Phytase[2], FTU/kg	AID Ca[3]		AID P[3]	
		7 to 9d[4]	19 to 21d[4]	7 to 9d	19 to 21d
6.5	0	0.59^{de}	0.50^{abc}	0.35^{e}	0.48^{d}
	500	0.66^{cd}	0.59^{a}	0.63^{bc}	0.70^{abc}
	1000	0.73^{ab}	0.59^{a}	0.75^{a}	0.77^{a}
8.0	0	0.53^{e}	0.48^{bc}	0.28^{e}	0.39^{e}
	500	0.68^{bcd}	0.58^{ab}	0.58^{cd}	0.69^{bc}
	1000	0.73^{abc}	0.51^{abc}	0.73^{ab}	0.73^{ab}
9.5	0	0.58^{e}	0.59^{a}	0.26^{e}	0.39^{e}
	500	0.70^{abc}	0.44^{ab}	0.52^{d}	0.65^{bc}
	1000	0.77^{a}	0.45^{c}	0.73^{ab}	0.69^{c}
SEM		0.017	0.023	0.021	0.016
Main effect means					
Ca, g/kg	6.5	0.66^{ab}	–	0.58^{a}	0.64^{a}
	8	0.64^{b}	–	0.53^{b}	0.59^{b}
	9.5	0.68^{a}	–	0.51^{b}	0.57^{b}
	SEM	0.01		0.012	0.009
Phytase, FTU/kg	0	0.56^{c}	–	0.29^{c}	0.41^{c}
	500	0.68^{b}	–	0.58^{b}	0.67^{b}
	1000	0.74^{a}	–	0.74^{a}	0.72^{a}
	SEM	0.01		0.012	0.009
Main effect and interaction P-values					
Ca		0.03	< 0.01	< 0.01	< 0.01
Phytase		< 0.01	0.62	< 0.01	< 0.01
Ca × Phytase		0.35	< 0.01	0.27	0.16
Orthogonal contrast					
Ca linear		0.1	< 0.01	< 0.01	< 0.01
Ca quadratic		0.03	0.97	0.45	0.23
Phytase linear		< 0.01	0.71	< 0.01	< 0.01
Phytase quadratic		0.05	0.43	< 0.01	< 0.01

[a–c]Least square means within a column with different superscript letters differ ($P < .05$).
[1] n = 8. Starting BW was 147 ± 1 and 697 ± 21 d/bird for birds at 7 (fed from 7 to 9 d) and 19 d (fed from 19 to 21 d) of age, respectively.
[2] Formulated concentrations, analyzed concentrations are shown in Table 2.
[3] Coefficient of AID Ca or P was calculated as: AID = $[(Min/TiO_2)_d - (Min/TiO_2)_i]/(Min/TiO_2)_d$. $(Min/TiO2)_d$ is the ratio of minerals (Ca or P) to TiO_2 in the diet and $(Min/TiO_2)_i$ is the ratio of minerals (Ca or P) to TiO_2 in the ileal digesta.
[4] 7 to 9 d, birds fed experimental diets from 7 to 9 d of age, 6 birds per replicate; 19 to 21 d, birds fed experimental diets from 19 to 21 d of age, 3 birds per replicate.

2.7. Statistical analysis

Data were analyzed by MIXED and REG procedures of SAS (SAS Institute, 2008). Treatment was considered as a fixed effect and pen for each age group as a random effect. To determine the dietary impact of Ca and phytase, the data were analyzed as 3 × 4 factorial design, by age. Orthogonal contrast was used to determine quadratic and linear responses of AID of Ca or P to Ca and phytase. Differences of AID Ca or P between 21 and 9 d old birds were regressed against analyzed Ca concentration and phytase activity in the diet to evaluate age effect. Tukey's (1949) adjustment was applied in all pair-wise comparisons to protect *P*-values. All calculations were generated based on pen averages, except phytase efficacy was calculated from treatment means. Significance was declared at $P < .05$.

3. Results

The basal diet analyzed lower than formulated (2.6 vs. 3.0 g/kg, respectively, Table 1) for P in the basal and thus P in Trt diets was lower than formulated (Table 2). Analyzed concentrations of Ca were all close to formulated values (Table 2). Phytase activities in non-phytase Trts were all below the detection limit, suggesting no cross-contamination among phytase and non-phytase Trt (Table 2). Analyzed phytase activities in phytase Trts were within 10% of formulated values, except in the 0.65% Ca containing 500 FTU phytase/kg, where the analyzed phytase concentration was 13% higher than the formulated value.

At 9 d of age, there was no interaction between Ca and phytase on either Ca or P digestibility (Table 3; $P > .05$). The AID P was decreased by 13% when diet Ca was increased from 6.5 to 9.5 g/kg (digestibility coefficient was 0.58 and 0.51, respectively; $P < .01$). Response of AID Ca to Ca concentration was different compared to AID P, where the lowest AID Ca was seen with 8.0 g/kg Ca (0.64 digestibility coefficient). Both AID Ca and AID P were increased at 9 d of age when phytase was added at 500 and 1000 FTU/kg. Across all Ca concentrations, AID Ca and P increased by 32 (from 0.56 to 0.74) and 155% (from 0.29 to 0.74), respectively, when 1000 FTU phytase/kg was added to the diet as compared to those fed diets without phytase inclusion ($P < .05$). At 21 d of age, an interaction between Ca and phytase was observed only in AID Ca ($P < .05$). The impact of Ca and phytase on AID P at 9 and 21 d of

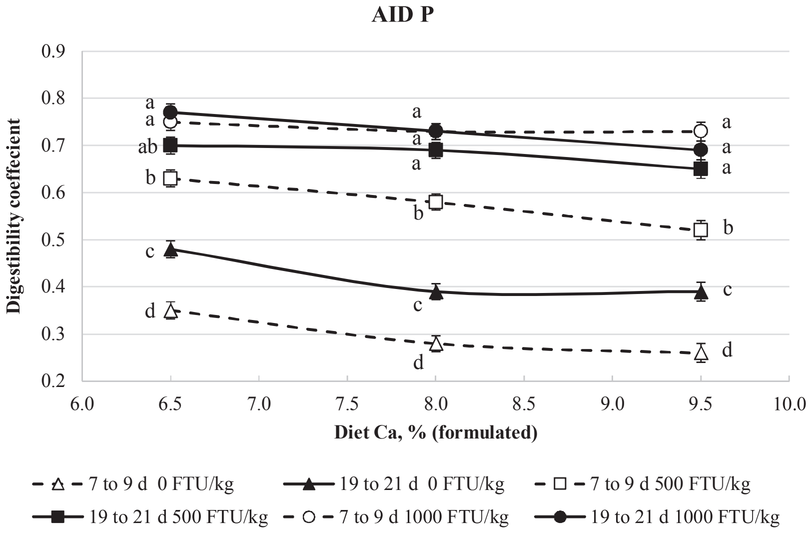

Fig. 1. Impact of age on P digestibility in response to Ca concentration and phytase dose (mean ± SEM). 7–9 d, birds fed experimental diets from 7 to 9 d of age, 6 birds per replicate; 19–21 d, birds fed experimental diets from 19 to 21 d of age, 3 birds per replicate.
[a–d]Least square means within a Ca concentration with different superscript letters differ ($P < .05$).

age were similar. Increasing Ca from 6.5 to 9.5 reduced AID P by 11%, while phytase inclusion increases AID P by 63 and 76% when 500 and 1000 FTU/kg were added ($P < .05$).

The degree of Ca and phytase's impacts on AID P differed between ages (Fig. 1). In the absence of phytase AID P was reduced in response to increased Ca concentration to a greater extent in younger as compared to older birds, whereas in the presence of phytase, differences in AID P were smaller between younger and older birds, especially at high Ca concentration. The age effect of Ca and phytase on the coefficient of AID Ca (Fig. 2) was different depending on phytase inclusion. With no phytase inclusion there is no effect of Ca on the coefficient of digestibility in younger birds as compared to older birds.

4. Discussion

The impacts of Ca and phytase on AID P have been well established and are in agreement with findings from current trial (Angel et al., 2002; Tamim et al., 2004; Selle and Ravindran, 2007; Li et al., 2014; Li et al., 2016). The discussion will therefore focus primarily on the age effect of the response difference between 9 and 21 d old birds.

In order to identify the age effect, feeding duration needs be carefully selected so as to avoid homeostatic changes in digestive and

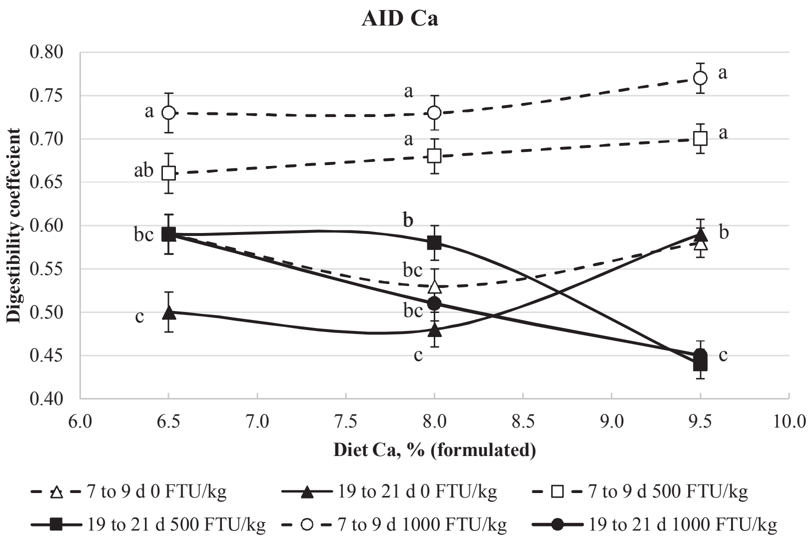

Fig. 2. Impact of age on Ca digestibility in response to Ca concentration and phytase dose. 7–9 d, birds fed experimental diets from 7 to 9 d of age, 6 birds per replicate; 19–21 d, birds fed experimental diets from 19 to 21 d of age, 3 birds per replicate.
[a–d]Least square means within a Ca concentration with different superscript letters differ ($P < .05$).

absorptive capacity that can impact Ca and P digestibilities under Ca and or P deficiency and Ca to P imbalance conditions. For example, 30 h feeding duration was used to determine the impacts of diet Ca and micro-mineral source on PP hydrolysis in 21 d old broiler birds (Tamim and Angel, 2003); effects of Ca and phytase on PP disappearance, P utilization and phytase efficacy in 23 d old broiler birds (Tamim et al., 2004). In another study, with a similar approach to that of Tamim et al. (2004), the experimental duration was 24 h to determine Ca and phytate interactions in broilers at 17 d of age (Plumstead et al., 2008). A series of trials conducted in our lab have suggested that the ideal time to determine dietary impacts on AID P and Ca should be no more than 48 h after the start of feeding deficient or imbalance Ca and P diets to minimize homeostatic physiological adaptations and that no fasting period is recommended or needed (Proszkowiec-Weglarz et al., 2013; Proszkowiec-Weglarz and Angel, 2013).

In the absence of phytase, AID P was in general greater in older birds irrespective of Ca concentration, but difference in AID P between 9 and 21 d old birds were greater as Ca increased (Fig. 1). These differences showed that younger birds were less capable of digesting P and more susceptible to high Ca concentration and/or Ca P imbalance when diets severely deficient in nPP were fed. This lower capacity to digest P can be partly due to the overall poorer digestive tract development in young birds, where lower digestive enzyme activities and villi heights associated with poorer nutrient digestion and absorption have been reported as compared to older birds (Obst and Diamond, 1992; Uni et al., 1998, 1999; Batal and Parsons, 2002).

With regard to P utilization, a similar age effect on P retention was reported in birds fed P deficient and sufficient diets (Olukosi et al., 2007). A more recent trial compared the effect of age on phytate degradation in different gastro-intestinal segments and found significantly lower phytate hydrolysis in 4 d old birds as compared to 14 d old birds in gizzard, jejunum and ileum (Morgan et al., 2015). However, it should be noted that, in both trials discussed above, animals were fed for more than 7 d on the experimental diets. This different approach could result in, potentially, confounding effects due to physiological adaptations, with upregulation in nutrient intake especially under P deficiency or Ca/P imbalance conditions (Yan et al., 2007; Proszkowiec-Weglarz et al., 2013; Proszkowiec-Weglarz and Angel, 2013), minimizing actual age effects seen in those trials.

Among all the different gastro-intestinal tract segments, the gizzard is likely to be the most important organ regulating feed passage (Svihus et al., 2004). A longer residence time in the gizzard has been suggested to be associated with better digestion and absorption (Amerah et al., 2007). Lack of sufficient phytate hydrolysis in the gizzard may therefore lead to a greater impact of Ca on intestinal phytate utilization where Ca forms precipitants with undigested phytate and prevents intestinal phytase to act (Applegate et al., 2003). Because shorter residence time has been reported in younger birds as compared to older ones (Ferrando et al., 1987; Van der Klis et al., 1990; Hetland and Svihus, 2001), insufficient phytate hydrolysis can be reasonably expected, which consequently could lead to greater detrimental effect of Ca on AID P as seen in the current study.

Even though the detrimental impact of Ca was seen regardless of phytase or age, it is, however, evident that inclusion of phytase changed the degree of the Ca impact on AID P especially at higher phytase dose (1000 FTU phytase/kg) as well as (9 d of age). With phytase inclusion, improvement in AID P in younger birds was greater than older birds, and the age-related difference in AID P disappeared when 1000 FTU phytase/kg was added to the diet. Using a similar approach as that of the current trial, Li et al. (2016) reported that, when birds were fed diets containing various concentration of Ca and PP from 11 to 13 d of age, average reduction of inositol hexakisphosphate (IP6) in gizzard was 44 and 62% when a *Buttiauxella* phytase was added to the diet at 500 and 1000 FTU/kg, respectively. As a result, ileal IP6 degradation was increased from an average of 0.20 with no added phytase to 0.86 with 1000 FTU phytase/kg inclusion. Their results indicate that quick and complete removal of IP6 in upper tract can facilitate optimum P utilization in the small intestine. In younger birds that do not have fully developed intestinal function and have relative rapid passage rate, the use of exogenous phytase may exert a greater benefit, in terms of maximizing P utilization and minimizing the detrimental effect of Ca, as compared to those at older age. In addition, the difference in Ca requirement between 9 and 21 d of age birds and the different Ca/P ratios tested may be another possible explanation of the greater degree of improvement seen in younger birds especially at 9.5 vs. 6.5 g/kg Ca.

Compared to the interest in P utilization, relatively less emphasis has been placed on Ca utilization in response to phytase addition. This, in part, is because Ca has been considered as a relatively cheap ingredient and its interactions with P and phytase partially disregarded. The limited amount of work that has been done to understand the effect of phytase on Ca digestibility, resulted in inconclusive and contrary results (Yi et al., 1996; Onyango et al., 2005; Adeola, 2010; Rutherfurd et al., 2012; Walk et al., 2012; Amerah et al., 2014). Ravindran et al. (2006) showed that when a diet containing 1000 FTU *E.coli* phytase/kg (.75% Ca and 0.25% nPP) was fed to broiler chickens from 14 to 21 d of age, along with the improved AID P as a result of phytase addition (64.6 vs. 51.6%; phytase vs. non-phytase Trt), AID Ca was 19% higher than that in birds fed non-phytase diets (41.7 vs. 34.9%). Similar improvements in AID Ca were also reported by others (Onyango et al., 2005; Adeola, 2010; Rutherfurd et al., 2012). In contrast, limited impact of phytase on AID Ca was reported (Yi et al., 1996; Walk et al., 2012; Amerah et al., 2014).

It is not unexpected to see the inconsistent responses to diet Ca and phytase on AID Ca in the current trial. In the presence of phytase, increased AID Ca in younger birds was seen regardless of Ca level, while the impact of phytase was much less at 21 d of age (Table 3), resulting a greater difference in AID Ca between 9 d and 21 d old birds (Fig. 2). To our knowledge, this age effect of phytase on AID Ca has not been discussed previously in broiler chickens. A study in swine showed that phytase was only able to improve AID Ca in young pigs (less than 40 kg), but failed to improve AID Ca in older pigs of between 60 and 100 kg (Kemme et al., 1997). This, in part, supports our finding that phytase was more effective in improving Ca digestibility in younger vs. older birds.

Phytase efficacy was expressed as net improvement of DigP, and calculated by difference between DigP documented for broilers fed the same diet with or without phytase at same age. Even though phytase efficacy was a calculated value from treatment average, it is evident that 1000 FTU phytase/kg was more effective in improving AID P in younger than older birds (Fig. 3).

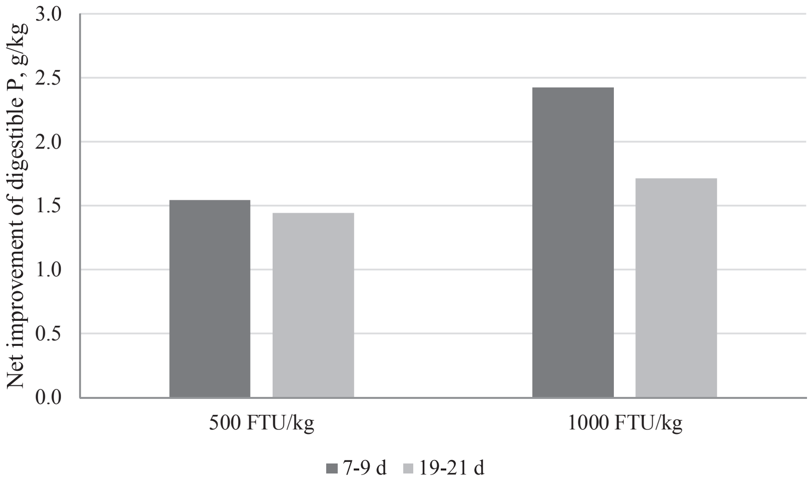

Fig. 3. Impacts of age on phytase efficacy. Phytase efficacy was calculated as: Dig P increase (g/kg) = (AID P_{phy} − AID $P_{non\text{-}phy}$) × P_{diet}. Dig P increase, net increase in digestible P; AID P_{phy}, AID $P_{non\text{-}phy}$, P digestibility in birds fed phytase and non-phytase diets, respectively; P_{diet}, analyzed total P concentration in the diet. 7–9 d, birds fed experimental diets from 7 to 9 d of age, 6 birds per replicate; 19–21 d, birds fed experimental diets from 19 to 21 d of age, 3 birds per replicate.

5. Conclusion

Impacts of Ca and phytase on P digestibility have been examined extensively in the past two decades. However, most of the studies were conducted in broilers around 21 d of age, and did not test for an age effect especially with that in young birds where digestive tract still is not fully developed and passage rate is faster. Without fully understanding the impact of age on phytase efficacy and Ca impact, it is difficult to optimize phytase efficacy and PP utilization as well as minimize use and dependency on inorganic P. The current study for the first time, examined age dependent response to Ca and phytase, and found younger animals were more susceptible to the detrimental effect of Ca. The results also suggested that using a high dose of phytase to help degrade phytate as much as possible in the upper digestive tract (up to and including the gizzard) could significantly alleviate the Ca impact and maximize P digestibility.

Conflicts of interest

None.

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